Genetic Analysis of Leaf and Stripe Rust Resistance in the Spring Wheat (*Triticum aestivum* L.) Cross RL4452/AC Domain

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ABSTRACT

Leaf rust and stripe rust of wheat (*Triticum aestivum* L.) are caused by the fungal pathogens *Puccinia triticina,* and *Puccinia striiformis* f.sp. *tritici,* respectively. In North America, the incorporation of adult-plant resistance (APR) genes into breeding lines has been an important strategy to achieve durable resistance to both diseases. Previously, the spring wheat cultivar AC Domain was reported to express an effective level of adult-plant resistance (APR) to leaf rust under field conditions. Early gene postulation work had suggested AC Domain might carry the APR gene *Lr34* due to its phenotypic similarity to other *Lr34* carrying lines. However, new gene specific markers have shown that AC Domain is not a carrier of *Lr34*. The objective of this research was to genetically localize the resistance in AC Domain, which is important because the cultivar has frequently been used as a parent in Canadian breeding programs, primarily for its value as a source of pre-harvest sprouting resistance. A mapping population of 185 doubled haploid (DH) lines derived from the cross 'RL4452' by 'AC Domain' was used for this study. RL4452 is a known carrier of *Lr34*. During 2011-2012, the DH population was evaluated in field leaf rust nurseries at Saskatoon, SK and Portage, MB and at a stripe rust nursery at Lethbridge, AB. Field results indicated that rust resistance in the mapping population was variable, with lines ranging from highly resistant, to highly susceptible. DH lines carrying *Lr34* showed a high level of resistance to both diseases. Thus, the non-*Lr34* carriers were genotyped using select SSR markers, and by an Illumina 9k Infinium iSelect SNP assay for subsequent quantitative trait loci (QTL) analysis. QTL analysis revealed that AC Domain donated a major resistance QTL located on chromosome 2BS, that mapped 46 cM proximal to markers linked to *Lr16,* and explained a significant portion of the leaf and stripe rust phenotypic variance in all test environments. In addition, this QTL was significantly associated with the expression leaf tip necrosis (LTN), reduction in area under the disease progress curve (AUDPC), and coefficient of infection (CI). In certain environments the interaction between the 2B QTL and *Lr34* was additive resulting in a superior level of rust resistance. Indoor rust testing showed AC Domain was susceptible to both diseases at the seedling stage. Taken together these results suggest that the identified resistance in AC Domain is likely due to the presence of an APR gene, on chromosome 2BS.

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1.0 INTRODUCTION

Leaf and stripe rust, caused by the fungal pathogens *Puccinia triticina* Eriks. (Formerly *P. recondita* f. sp. *tritici Rob. ex Desm.)*, and *P. striiformis* Westend. f. sp. *tritici* Eriks., respectively, are two important foliar diseases of wheat (*Triticum aestivum* L.) in western Canada (Bailey et al. 2003) and globally. Both pathogens share similarities in their life-cycles and biology, but differ in their environmental adaptation (Knott 1989). In Canada, *P. triticina* is the most common and widespread of the cereal rust pathogens, and is one of the most prevalent pathogens of wheat worldwide. Rust infection can often result in significant yield losses to producers, by reducing the total number of kernels produced, and decreasing kernel weight (Bolton et al. 2008a). Leaf rust does not result in total crop failure, but yield losses of up to 40% can be incurred under severe epidemic conditions (Knott 1989). Stripe rust is regarded as a more destructive disease, and localized epidemics have resulted in up to 70% crop losses in some areas (Fetch et al. 2011).

Controlling wheat rust is most effectively achieved through resistance breeding (McIntosh 1992), which is considered an environmentally friendly and economically sound approach to prevent yield losses (Singh et al. 1998, Vida et al. 2009, Herrera-Foessel et al. 2011). Consequently, the maintenance and transfer of leaf rust resistance genes into regionally adapted cultivars has been a major objective of wheat breeding programs globally (McIntosh 1992, Spielmeyer et al. 2008). Studies have shown that breeding for disease resistance is not only important for producers, but can also have significant financial benefits to breeding programs. Marasas et al. (2003) examined the cost/benefit ratio of research inputs at CIMMYT, and found that there was a 27:1 return on research investment towards breeding for leaf rust resistance between the years of 1967-2007.

To date, the predominant target for breeders has been the incorporation of race-specific (also referred to as major gene, all-stage) resistance into new cultivars (McIntosh et al. 2008). Race-specific resistance is expressed at all stages of plant development (Lagudah 2011) and is typically controlled by a single gene that confers a hypersensitive response to prevent further invasion by the rust pathogen (Mateos-Hernandez et al. 2006, Rosewarne et al. 2006). Presently, over 50 resistance genes have been identified and catalogued for each disease in wheat, the majority of which are considered to be race-specific (McIntosh et al. 2008). In addition, a second

type of resistance exists in wheat commonly referred to as adult-plant resistance (APR). In contrast to major gene resistance, APR is only expressed at the adult-plant phase of the wheat life cycle (William et al. 2006) and functions by allowing infection to occur, but slowing disease development so that the infection is of reduced consequence to the plant. Because of the compatible infection type, this "slow-rusting" resistance has proven to be durable in the field (Mateos-Hernandez et al. 2006). In addition, some of the reported APR genes in wheat confer pleiotropic, broad-spectrum resistance to multiple biotrophic pathogens. To date, three such gene complexes have been identified: *Lr34/Yr18/Pm38*, *Lr46/Yr29/Pm39* and *Lr67/Yr46 (*Dyck et al. 1966, Singh 1992, Lillemo et al. 2008, Singh et al. 1998, William et al. 2003, Hiebert et al. 2010, Herrera-Foessel et al. 2011). All three APR genes/gene complexes confer dual resistance to leaf and stripe rust, and in addition, *Lr34* and *Lr46* are also associated with APR to powdery mildew (*Erysiphe graminis* f.sp. *tritici)*. Furthermore, all three genes/gene complexes are associated with the expression of the leaf tip necrosis (LTN) phenotype (Hiebert et al. 2010).

Lr34 is the most extensively studied APR gene, and it is estimated that as many as 50 percent of wheat cultivars carry it worldwide (Krattinger et al. 2011). *Lr34* has been cloned, and its gene product identified as an ATP-binding cassette (ABC) transporter (formerly pleiotropic drug transporter family) of the ABCG subfamily (Kratinger et al. 2009). There are an estimated 60 such coding regions spread throughout the wheat genome, but it is not yet known how many of these play a role in disease resistance (Krattinger et al. 2011). In contrast, it is not known if the defense mechanisms of *Lr46* and *Lr67* are similar to that of *Lr34*, and their map locations are poorly defined. In addition, the cumulative phenotypic effect of combining more than one APR gene in a cultivar is poorly understood, although it has been suggested that combining APR genes could result in increased resistance and durability in the field. For example, Singh et al. (2000a) have shown that combining four to five APR genes confers near immunity to rust infection. However, a paucity of information exists regarding specific combinations of APR genes that provide the best level of resistance, highlighting an important area for future research (Lagudah 2011). Another important strategy commonly used by breeders had been the stacking of resistance genes with different resistance mechanisms, such as APR and seedling resistance (Rubiales and Niks 1995).

The spring wheat cultivar AC Domain has expressed a strong level of APR to leaf rust over 10 years of field testing in Manitoba (Brent McCallum, personal communication). Based on

phenotypic similarity with lines known to carry *Lr34,* AC Domain was hypothesized to carry *Lr34* (Liu and Kolmer 1997). However, an understanding of allelic variation and the development of gene specific molecular markers for *Lr34* has now shown that AC Domain does not carry *Lr34* (McCallum et al. 2008). AC Domain carries the defeated seedling gene *Lr10* and the partially effective seedling gene *Lr16 (*McCartney et al. 2005b), but neither of these genes would be expected to confer the APR typical of that observed in AC Domain. It is now hypothesized that AC Domain likely carries additional APR gene(s), one of which could be *Lr46* (Brent McCallum, personal communication). Determining the basis of the leaf rust resistance in AC Domain is important because it has been frequently used as a parent in Canadian breeding programs (McCallum et al. 2012a).

1.1 Project Hypothesis

Based on the literature reviewed, the following hypotheses were developed:

1. The APR resistance gene found in AC Domain is *Lr46*;
2. When combined, *Lr34* and the APR resistance gene in AC Domain (*Lr46)* have an additive effect against leaf and stripe rust.

1.2 Project Objectives

The main goal of this research was to localize the APR in AC Domain, and to determine if the combined effects of two APR genes enhance leaf and stripe rust resistance in hexaploid wheat. Based on the above hypotheses, the following were the objectives of this thesis:

1. Examine a population of 185 lines derived from the cross RL4452/ 'AC Domain' for leaf and stripe rust reaction;
2. Genetically localize the resistance in AC Domain (*Lr46*);
3. Determine if *Lr34* and the second APR gene (*Lr46)* show additive effects for leaf rust and stripe rust resistance.

2.0 LITERATURE REVIEW

2.1 Wheat Biology and Production in Canada

In 2012, over 9.6 million hectares were seeded to wheat in Canada, accounting for the highest acreage of any crop (Statistics Canada, 2012). The two most commonly grown wheat species are hexaploid bread wheat (*T. aestivum*, 2n=6x=42, genome AABBDD), and tetraploid durum wheat (*T. turgidum* L. var. *durum*, 2n=4x=28, genome AABB) (Knott 1989). The Canada Western Red Spring (CWRS) bread wheat market class represents the most widely grown of all wheat market classes, accounting for 6.4 million hectares of the total Canadian wheat production in 2012 (Statistics Canada, 2012). CWRS wheat cultivars obtain a premium price in the world market, which has led to the high production area (McCallum and DePauw 2008). In addition, CWRS wheat has superior milling characteristics and can be used in a wide range of end use products (McCallum and DePauw 2008). The primary use for durum wheat is for milling into semolina, which is used primarily for pasta and couscous. Durum wheat accounts for approximately 5% of Canadian wheat production, and in 2012, was grown on 1.9 million hectares (Statistics Canada, 2012).

2.2 Generalized Rust Life Cycle

Wheat rusts are biotrophic organisms of the phylum *Basidiomycota*, order *Uredinales* that can only survive on a living host plant (Zhang et al. 2003). Leaf and stripe rust are heteroecious and macrocyclic, and have five distinct spore stages, requiring two different hosts to complete their full life cycle (Bolton et al. 2008a). Members of the wheat family (*Triticum* spp.) are considered the primary hosts of leaf and stripe rust (Table 1). On wheat, rust can only produce asexual urediniospores, basidiospores and teliospores, the latter being the overwintering stage of the fungus. The sexual phase of the *P. triticina* and *P. striiformis* life cycle can only occur on an alternate host, for which each pathogen has a different preference.

Table 1: Host range for leaf rust and stripe rust.

Leaf Rust (*Puccinia triticina*)	**Stripe Rust (*Puccinia striiformis* f. sp. *tritici*)**
Primary Hosts *T. aestivum*[1] *T. turgidum* L. var. *durum*[1] *T. dicoccoides* (Wild emmer)[1] *Aegilops speltoides*[1] *Triticosecale*[1]	**Primary Hosts** *Triticum*[3] species *Hordeum vulgare*[3] *Secale cereale*[3] *Triticosecale*[3] – some genotypes
Alternate Hosts: *Thalictrum speciosissimum*[1] *Isopyrum fumaroides*[1] *Anchusa*[2], *Anemonella*[2], *Clematis*[2]	**Alternate Host:** *Berberis holstii* [6] *Berberis vulgaris* [6] *Berberis chinensis* [6] *Berberis koreana* [6]

[1]Bolton et al. 2008a, [2]Wiese 1987, [3]Wellings 2007, [4]Bailey et al. 2003, [5]Walker et al. 2011, [6]Jin et al. 2010.

In North America, leaf and stripe rust infection occurs primarily as the result of a continuous asexual urediniospore infective cycle. This is because both urediniospore production and infection occurs only on wheat. Urediniospores are single celled and dikaryotic, and are released from uredinia for a period of a few weeks (Knott 1989). Depending on wind speed, urediniospores most frequently travel short distances causing localized re-infection, but can also be transported long distances helping the disease spread over large geographical areas (Knott 1989). Once urediniospores land on the leaf, germination occurs within three hours if favorable environmental conditions exist (Roelfs et al. 1992, Bolton et al. 2008a). Adaptation to different environmental conditions is another key difference between leaf and stripe rust. The optimal temperature range for germination of urediniospores of *P. triticina* is between 10 and 25℃. In contrast, the optimum temperature for *P. striiformis* is 11℃ (Roelfs et al. 1992), and the highest rate of urediniospore germination occurs between 0 to 15℃, with a maximum limit of 21℃ (Wiese 1987).

Once germination has begun, the newly emerged germ tube will grow towards the stomata over which it will form an appressorium (Roelfs et al. 1992). The appressorium then forms a penetration peg, which is used to push through the closed stomata, allowing entry into the leaf (Bolton et al. 2008a). Once inside the leaf, infective hyphae will spread throughout the intercellular space and form haustorial mother cells (HMCs) beside leaf mesophyll, or epidermal cells (Bolton et al. 2008a). Generally, HMC formation occurs between 12 to 24 hours following penetration (Hu and Rijkenberg 1998). In the case of a compatible disease reaction, a haustorium will differentiate from each HMC to form a round-bodied structure with a tubular neck band known as the haustorium (Hu and Rijkenberg 1998). The haustorium is a specialized parasitic feeding structure, which the fungus uses to obtain nutrients from the living host cell (Hu and Rijkenberg 1998). During an incompatible reaction, the host plant will "sense" infection, and initiate apoptosis of the infected cell that contains the haustorium, causing the haustorium to die (Roelfs et al. 1992).

The haustorium is not an intracellular structure, since it does not puncture the host cells plasma membrane, but rather forms an extra-haustorial membrane (EHM) derived from the host cells own plasma membrane (Panstruga 2003). During this phase of infection, few adverse effects can be observed in the susceptible host plant, which is typical of the first several days of the infection process (Bolton et al. 2008a). Under favorable conditions, the fungus will continue to spread within the leaf producing numerous haustoria, and approximately seven to ten days post inoculation (DPI) for leaf rust, and ten to 14 DPI for stripe rust, the first sign of uredinial development in the hyphae becomes evident (Bolton et al. 2008a, Wiese 1987). Shortly after, the plant cell wall will break, releasing masses of urediniospores (Bolton et al. 2008a). The production and subsequent release of urediniospores from the uredinium is a continual process (Eversmeyer and Kramer 2000). However, the peak urediniospore release occurs approximately four days following the first sporulation event (Roelfs et al. 1992).

Teliospores are the overwintering body of the rust fungus and are produced by fruiting structures called telia. During teliospore formation, karyogamy occurs between two haploid nuclei to form diploid nucleus (Bolton et al. 2008a). The diploid nucleus of each teliospore will subsequently undergo meiosis to form a promycelium containing four haploid nuclei. Each of these haploid nuclei enters a newly forming basidiospore. After a single mitotic division, the end result is four basidiospores each containing two identical nuclei (Bolton et al. 2008a).

Basidiospores lack the ability to re-infect wheat, therefore require an alternate host to complete the sexual phase of the life cycle (Bolton et al. 2008a). Basidiospores must travel by air to reach the alternate host, upon which they germinate to infect the host epidermal cells where it produces specialized mating structures known at pycnia on the upper surface of the leaf (Bailey et al. 2003). Pycnia consist of two different mating types (+/-), and give rise to pycniospores. Rust is considered heterothallic because pycniospores are not capable of fertilizing the same pycnia from which they are produced (Bolton et al. 2008a). Once cross fertilization has occurred, fruiting structures known as aecia will form on the lower surface of the alternate host leaf, within which produced aeciospores are capable of re-infecting wheat (Bolton et al. 2008a).

The difference in requirement for the alternate host is one of the key differences between *P. triticina* and *P. striiformis* (Bolton et al. 2008a, Jin et al. 2010). Basidiospores of *P. triticina* can only infect certain species of the genera *Thalictrum*, *Isopyrum*, *Anchusa* and *Clematis* (Table 1). In Europe and Asia, *Thalictrum* is susceptible to *P. triticina*, and will produce spores capable of re-infecting wheat. In contrast, North American species of *Thalictrum* are highly resistant to *P. triticina*, and therefore will not usually become infected (Bailey et al. 2003). Until recently, no alternate host for *P. striiformis* had been identified and the disease was believed to exist only in the asexual form. However, new evidence proves that some species of the genus *Berberis* (Table 1) can serve as an alternate host for the pathogen (Jin et al. 2010). Interestingly, North American barberry eradication initiatives implemented to control the sexual reproduction of the stem rust (*Puccinia graminis* f. sp. *tritici*) pathogen may have also indirectly limited the sexual reproductive cycle of *P. striiformis* populations.

2.3 Leaf Rust (*Puccinia triticina*)

Of the three wheat rust pathogens, *P. triticina* is the most common, widespread and widely adapted pathogen (Bariana et al. 2007). Leaf rust is expected to have originated in the Middle East because it is the only region where the primary and alternate hosts naturally co-exist (Bolton et al. 2008a). In Canada, the disease can proliferate in all regions where wheat is grown (Bailey et al. 2003), but the most severe outbreaks usually occur in Manitoba and south-eastern Saskatchewan where environmental conditions are most conducive to infection and spread of the disease (McCallum et al. 2007). Infection often results in yield losses that range up to 20%, but may be as high as 40% during an epidemic (McCallum et al. 2007). The yield losses attributed to leaf rust come as a direct result of infection of the flag leaf (Eversmeyer and Kramer 2000),

which ultimately causes reduced floret-set, reduced kernel weight and reduced grain quality characteristics (Roelfs et al. 1992).

2.3.1 Epidemiology of Leaf Rust

Leaf rust travels to Canada via the "*Puccinia* pathway", which spans the 3000 kilometer region from Mexico to Canada (Knott, 1989). For three main reasons, leaf rust has the potential to develop into widespread epidemics in North America. First, there is a geographical connection where large adjacent fields extend from Mexico into the northern Great Plains. Second, the pathogen has the ability to rapidly reproduce asexually by producing large numbers of windblown urediniospores that are capable of travelling long distances (Eversmeyer and Kramer 2000). Lastly, the overlap in growth stages between winter and spring seeded crops along the *Puccinia* pathway allows the disease to overwinter and spread sequentially from mature, to newly emerging plants (Kolmer et al. 2007). Volunteer wheat infected with rust during the summer is also considered an important source of inoculum that initiates the disease into fall planted wheat crops (Kolmer et al. 2007). The disease overwinters on wheat plants in Mexico and the southern United States. By February, the disease begins to spread north, through the production and release of asexual urediniospores on newly maturing wheat crops (Bailey et al. 2003). The disease proliferates on winter wheat crops throughout the southern Great Plains and south-eastern United States, and by late May symptoms may be observed on winter wheat crops in the northern Great Plains (Kolmer et al. 2007). Infection then passes from winter, to spring wheat (Kolmer et al. 2007). Symptoms will typically appear in western Canadian fields as early as June, becoming widespread by late July and progress to a point of maximum severity near physiological maturity of wheat in August. It is during this time that *P. triticina* will begin to produce teliospores, the overwintering stage of the fungus (Bailey et al. 2003). In Canada, teliospores of *P. triticina* do not survive the winter (Bailey et al. 2003).

Leaf rust is well adapted to temperate areas, and epidemics are at risk of developing early in the season when the weather is cool around the time when winter wheat is breaking dormancy (Eversmeyer and Kramer 2000). The most important factor contributing to leaf rust epidemics is overwintering of the pathogen on live plants as uredinia, or as latent infection (Roelfs et al. 1992). According to Eversmeyer and Kramer (2000), the most important source of inoculum for western Canada is infected winter wheat. When urediniospore infection occurs on winter wheat in the fall, the infection process can progress to an advanced stage before the winter crop

becomes dormant. Once dormancy breaks in the spring, the pathogen has a head-start, allowing for maximum infection in that location (Eversmeyer and Kramer 2000).

2.4 Stripe Rust (*Puccinia striiformis*)

Stripe rust (yellow rust) is caused by the fungal pathogen *P. striiformis* Westend. f. sp. *tritici* Eriks. (McIntosh 1992). On a global scale stripe rust is relatively widespread with localized epidemics occurring in more than 60 countries (Chen 2005). In Canada, stripe rust is of primary concern to wheat production in central and southern Alberta, where in recent years the disease is thought to have overwintered (Kumar et al. 2012). Symptoms of stripe rust include characteristic striped uredinia on the leaf surface arising from a single urediniospore infection (Knott 1989). In a severe epidemic, stripe rust can be as destructive as stem rust (Roelfs et al. 1992), causing yield losses up to 70% (Fetch et al. 2011). Similar to leaf rust, the most effective way to control stripe rust is through the use of resistant cultivars (Chen 2005). The disease differs from leaf rust in that it is better suited to cooler temperatures typical of northern temperate climates, higher elevations or on crops grown during the winter season in tropical climates (Knott 1989). In North America, stripe rust can be prolific in the Pacific Northwest region of the United States, and other areas that have cool night temperatures, frequent light rainfall and regular dew periods (Chen 2005). This is a concern to Canadian wheat production because stripe rust spores are believed to blow into Canada from the Pacific Northwest region (Chen 2005). Another concern with respect to western Canada is that prevalence of diverse races of *P. striiformis* in the Pacific Northwest region of the United States may be due to the continued sexual reproduction of *P. striiformis* on barberry in the region (Jin 2010). The optimal temperature required for stripe rust infection is approximately 11ºC (Wiese 1987). Fortunately, the environmental conditions required for stripe rust to flourish limits the distribution and spread of the disease (Roelfs et al. 1992). However, recent evidence has shown that stripe rust populations may be adapting to warmer temperatures, making the disease a serious threat to Canadian wheat production (Milus et al. 2009). Another risk factor is that stripe rust can overwinter in southern Alberta during mild winters leading to early disease development in the spring (Sanford and Broadfoot 1932, Conner et al. 1988).

2.5 Control of Wheat Rusts

The most effective and environmentally sustainable method of controlling wheat rust is through the transfer of resistance genes into modern cultivars (Fetch et al. 2011). Other control

methods can include application of fungicides, and avoidance through early-seeding practices (McCallum et al. 2007). However, fungicides can be costly for producers and hazardous to the environment (Singh et al. 2000b). In the case of stripe rust, early seeding has been effective, but with evidence of overwintering of the pathogen in western Canada, this strategy may become increasingly ineffective. Quarantine is ineffective against rusts because airborne urediniospores can travel long distances (Roelfs et al. 1992). A goal in breeding programs should be to screen germplasm for durable resistance genes, and then attempt to combine them in a cultivar for long-term durable resistance (McCallum et al. 2007).

2.6 Rust Resistance Genes in Wheat

2.6.1 Major Resistance Genes

Presently there are over 50 major (all-stage, race specific) resistance genes for each cereal rust disease that have been identified in wheat (McIntosh et al. 2008). In the wheat-rust pathosystem, major gene resistance generally follows the traditional gene-for-gene hypothesis (Cloutier et al. 2007). It is believed that host resistance (*R*) genes encode receptors, which are only capable of recognizing specific pathogen effector molecules. Effector molecules are encoded by a corresponding avirulence gene (*Avr*) in the pathogen (Cloutier et al. 2007). When a host *R*-gene product recognizes the target *Avr*-gene product (either directly or through an intermediary protein), the incompatible reaction elicits a hypersensitive response in the host. To date, only three race-specific genes for rust have been cloned in wheat: *Lr1* (Cloutier et al. 2007*), Lr10* (Feuillet et al. 2003) and *Lr21* (Huang et al. 2003), all of which encode nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins. Although the physiological mechanism of resistance has not been reported for these three genes, it is likely that they operate in a similar fashion to what has been reported in other pathosystems. For example, in the flax (*Linum usitatissimum*) - flax rust (*Melampsora lini*) pathosystem, this same class of resistance protein has been demonstrated to recognize specific pathogen effector molecules eliciting a hypersensitive reaction (Keller et al. 2012). Although race-specific resistance is highly effective, changes in the pathogen population can quickly lead to resistance breakdown (William et al. 2003). This occurs when selection acts upon the pathogen population that has undergone mutation, migration and genetic recombination, sometimes resulting in a shift in virulence (Singh et al. 2000b). For example, in Mexico, single gene, race-specific resistance can usually be

overcome by the pathogen in less than three years under large-scale commercial deployment (Singh and Huerta-Espino 2003).

There have been several examples where the breakdown of effective, race-specific resistance has occurred. Recently, there have been reports of the detection of isolates virulent to *Lr21* in southern Manitoba (McCallum et al. 2012b), Minnesota, and North Dakota (Kolmer and Anderson 2011), which highlights potential vulnerabilities in North American germplasm. The major resistance gene *Lr21* was first introgressed into hexaploid wheat from *Aegilops taushii* (Rowland and Kerber 1974), and prior to 2010, conferred effective resistance to all leaf rust isolates in North America (Kolmer and Anderson 2011). In Canada, *Lr21* was first released in the cultivars AC Cora, McKenzie, Lovitt, and CDC Alsask (McCallum and Depauw, 2008). Because of its previous effectiveness against all pathogen races, *Lr21* has been heavily relied upon. In 2010, 50% of the wheat acreage in North Dakota and Minnesota was dependent on *Lr21* (Kolmer and Anderson 2011). *Lr16* is another example of a gene that is present in Canadian germplasm where increasing frequencies of virulent isolates have been reported (McCartney et al. 2005a). *Lr16* is still known to provide some protection against Canadian *P. triticina* populations, particularly in combination with additional resistance genes (German and Kolmer 1992). Canadian cultivars known to carry *Lr16* include AC Domain, AC Karma, AC Majestic, AC Splendor, Columbus and Grandin (McCartney et al. 2005a).

2.6.2 Multi-Pathogen APR genes in Wheat

APR genes are of great value to breeders because broad-spectrum resistance can be obtained by a single gene (Navabi et al. 2005). In contrast to race-specific resistance, APR is not associated with a hypersensitive reaction (McIntosh 1992) and is best expressed only during the adult phase of the plant life cycle (Rubiales & Niks 1995), although recent evidence has shown that expression can be induced under specific temperature and light combinations (Lagudah 2011). Another key feature of APR is that it provides resistance against a wide range of pathogen races (i.e. non-race specific resistance) which greatly contributes to its durability (Lagudah et al. 2006). APR genes confer a slow-rusting form of resistance that remains highly durable for long periods of time (Mateos-Hernandez et al. 2006, William et al. 2006, and Lagudah et al. 2009). Combining major and minor resistance genes in cultivars is thought to greatly improve durability and has been an important strategy in wheat breeding (McIntosh 1992, Rubiales and Niks 1995).To date, only three durable APR gene complexes have been identified in wheat and these

confer resistance to multiple biotrophic pathogens. These include *Lr34/Yr18/Pm38/Ltn1*, *Lr46/Yr29/Pm39/Ltn2*, and *Lr67/Yr46* (Table 2). In addition, all three loci are also associated with the expression of the leaf tip necrosis (LTN) phenotype, which has been used by breeders as a visual marker to identify lines that may carry APR.

The pyramiding of APR genes into cultivars is believed to result in highly durable, long-term resistance, but little is known about the benefits, interactions, or risks of such gene combinations (Hiebert et al. 2010). The combination of APR genes could provide higher levels of resistance than any one gene alone, as these genes are believed to act in an additive manner (Rosewarne et al. 2006). If this is the case, breeders could stack APR genes to breed for broad spectrum resistance to these wheat pathogens. Gene specific markers have been developed for *Lr34* (Table 3), which have been successfully deployed in plant breeding programs in Canada (Dakouri et al. 2010) and globally. However, the map locations of *Lr46* and *Lr67* are poorly defined limiting the potential for marker assisted breeding to combine these genes (William et al. 2006). Indeed the identification and development of robust molecular markers will play an important role in characterizing germplasm and subsequent APR gene transfer into new cultivars (William et al. 2006).

Table 2: Summary of previously described broad-spectrum APR genes in wheat.

Chromosome/Locus	**Leaf Rust**	**Stripe Rust**	**Powdery Mildew**	**Leaf Tip Necrosis**
7DS	*Lr34*[1]	*Yr18*[2]	*Pm38*[3]	*Ltn1*[4]
1BL	*Lr46*[5]	*Yr29*[6]	*Pm39*[7]	*Ltn2*[4]
4DL	*Lr67*[8]	*Yr46*[9]	Unknown	Unnamed

[1] (Dyck et al. 1966), [2] (Singh 1992), [3] (Lillemo et al. 2008), [4] (Rosewarne et al. 2006), [5] (Singh et al. 1998), [6] (William et al. 2003), [7] (Lillemo et al. 2008), [8] (Hiebert et al. 2010), [9] (Herrera-Foessel 2011)

2.6.2.1 *Lr34/Yr18/Pm38/Ltn1*

The *Lr34* gene, formerly *Lrt2* (Dyck and Samborski 1982), was the first APR gene identified for its effect against leaf rust in wheat and is the most extensively studied APR gene in wheat globally. The gene has been localized to chromosome 7D (Dyck 1987), and has recently been cloned and sequenced (Krattinger et al. 2009). *Lr34* has been described as being the most effective of the APR genes (Singh and Rajaram 1992, Lillemo et al. 2008). In addition to conferring resistance to leaf rust, *Lr34* pleiotropically confers APR to other biotrophic pathogens (Krattinger et al. 2009, Table 2), for which each has been given its own gene designation. These include stripe rust (*Yr18,* Singh et al. 1992), and powdery mildew (*Pm38,* Lillemo et al. 2008). Another interesting feature of *Lr34* is its association with the expression of leaf tip necrosis (LTN), which usually manifests 1-2 weeks after flowering (Lillemo et al. 2008). This has led to designation of the gene *Ltn1* (Lillemo et al. 2008). The LTN phenotype has been used as a phenotypic marker by breeders to select for APR. However, the severity of LTN is strongly influenced by environment, and may also be affected by the additive action of *Lr34* and other APR genes (Navabi et al. 2005). *Lr34* has been shown to enhance resistance to stem rust (*Puccinia graminis* f. sp. *tritici*) in some genetic backgrounds (Dyck and Samborski 1982). Dyck (1987) showed that near isogenic Thatcher-*Lr34* lines had greater stem rust resistance than Thatcher alone. In similar studies, Vanegas et al. (2008) confirmed that *Lr34* was directly correlated with increased APR to stem rust in certain populations. Fine-mapping of the locus performed by Spielmeyer et al. (2008) on a population derived from a cross between 'Thatcher' and RL6058 provided further validation to the theory that this locus is associated with stem rust resistance. The authors suggest that *Lr34* enhances stem rust resistance by interacting with one or more unlinked genes thought to be present in the Thatcher background (Spielmeyer et al. 2008). In addition, *Lr34* may play an important role in enhancing the expression of seedling stem rust resistance genes and may work as an anti-suppressor of resistance (Vanegas et al 2008). *Lr34* has also been shown to enhance resistance of partially defeated race-specific resistance. For example, the combined effects of *Lr34* and *Lr16* can provide a greater level of resistance when compared to the effects of each gene on its own (German and Kolmer 1992). A better understanding of how *Lr34* interacts with other resistance loci could greatly impact breeding strategies.

Lr34 has been shown to work in an additive manner with other unknown APR genes in some genetic backgrounds (German and Kolmer 1992, Singh and Rajaram 1992). The yield loss

prevented by *Lr34* has proven to be substantial when compared to near isogenic lines lacking the gene (Singh and Huerta-Espino 1997). Lines carrying *Lr34* still become infected, but infection is reduced by up to 50% (Vida et al. 2009). However, the protection provided by *Lr34* alone may not be sufficient under high infection pressure (Singh and Huerta-Espino 1997).

Lr34 has recently been cloned from the wheat cultivar Chinese Spring and encodes a putatively functional ABCG transporter protein (Krattinger et al. 2009). The genomic sequence of *Lr34* contains 24 exons and spans 11,805 bp, which encodes a predicted protein of 1401 amino acids (Krattinger et al. 2009). There are two alleles commonly found in wheat, referred to as *Lr34sus-D* and *Lr34res-D*. The latter codes for a functional ABCG transporter, and is found in all lines expressing *Lr34*-based disease resistance (Krattinger et al. 2013). The functional *Lr34*-coded transporter has undergone two "gain-of-function" mutations; these include a 'TTC' deletion in exon 11 resulting in a deletion of phenylalanine residue, and a C/T SNP in exon 11 causing a tyrosine to histodine amino acid switch (Krattinger et al. 2011). Recent evidence suggests both "gain-of-function" mutations occurred after the diversification of hexaploid wheat approximately 8000 years ago (Krattinger et al. 2013).

At this time, the physiological basis of *Lr34* mediated resistance is still unknown. Though, it is clear that *Lr34* does not function in the same manner as conventional NBS-LRR proteins (Singh et al. 2010, Keller et al. 2012). The major differences between these two classes of resistance proteins is that the *Lr34*-encoded transporter is best expressed in the mature plant and suppressed in the juvenile stages of growth, does not involve pathogen recognition, and confers resistance to all pathogen races (Keller et al. 2012). One hypothesis is that the *Lr34* may be involved in transporting some type of an "anti-fungal" metabolite that accumulates in the leaf to create an environment that is less conducive to pathogen growth (Singh and Huerta-Espino 1997). An alternate hypothesis proposed by Messmer et al. (2000) is that *Lr34* conditioned resistance could be the result of a physiological change within the flag leaf leading to reduced pathogenicity of biotrophic pathogens. Both hypotheses could explain why resistance is associated with the development of necrotic leaf tips.

It has been demonstrated that *Lr34* increases the latent period between initial infection and uredinium development, and confers a non-hypersensitive resistance (Rubiales and Niks 1995; Singh and Huerta-Espino 2003; Bolton et al. 2008b). The resistance provided by *Lr34* is also attributed to early abortion of the urediniospore germ tube during penetration (Rubiales and

Niks 1995). The increased latent period has a negative-effect on disease development because it helps keep inoculum levels low early in the growing season (Singh and Huerta-Espino 2003). The gene reduces the rate at which haustoria form early in the infection process as a direct result of a decrease in the number of infective hyphae that are formed (Rubiales and Niks 1995). *Lr34* may also increase the transcription of defense proteins early in the infection process, which may explain the increased latent period (Bolton et al. 2008b). Some studies suggest that the gene is best expressed in the adult-plant at temperatures averaging 20℃ (Singh et al. 2007), but can also be induced at the seedling stage when exposed to cold temperatures (Rubiales and Niks 1995). Thus, *Lr34* may be particularly effective in Canada and other regions where temperatures are lower during the wheat growing season.

In the United States where *Lr34* has been widely deployed since the 1970s, no isolates of *P. triticina* have been able to overcome its resistance (Kolmer et al. 2007). *Lr34* is widespread in old and current wheat cultivars (Rubiales & Niks, 1995), in part because its benefits have probably been inadvertently recognized and bred into many cultivars within the last century (Dyck 1987). It is estimated that as many as 50% of modern cultivars carry *Lr34* worldwide (Krattinger et al. 2011). *Lr34* has been traced back to the Italian hard red spring wheat cultivars Mentana and Ardito (released in the early 1900's), and was shortly thereafter transferred into the historically popular cultivar Frontana (Kolmer et al. 2008). It was first believed that the resistance in Frontana was due to the presence of a major resistance gene *Lr13* (Dyck et al. 1966). However, Singh and Rajaram (1992) found that the resistance provided by *Lr13* had become ineffective in Mexico and has been ineffective in South America since 1967. They concluded that the durable long-term resistance found in Frontana was more likely to be attributed to *Lr34* in combination with the additive interaction between at least three other APR genes (Singh and Rajaram 1992).

In Canada, combinations of *Lr34* with various seedling resistance genes have provided the most long-term effective leaf rust resistance (Kolmer and Liu 2002). The percentage of CWRS cultivars that carry *Lr34* has been on the rise since the late 1980's (McCallum et al. 2012a). In fact, prior to 1987, no Canadian cultivars carried *Lr34*, but since 2009, approximately 40% of the seeded area was to *Lr34*-carrying CWRS cultivars (McCallum et al. 2012a). Lines carrying such gene combinations often show a greater level of resistance than lines carrying race-specific genes alone (Kolmer and Liu 2002).

2.6.2.2 *Lr46/Yr29/Pm39/Ltn2*

Singh et al. (1998) identified a second APR gene, *Lr46* on chromosome 1BL of the wheat cultivar Pavon 76, which has likely contributed to its durable leaf rust resistance since its release in 1976. The leaf rust resistance conferred by *Lr46* is similar to *Lr34* in that it involves a compatible, non-hypersensitive reaction (Martinez et al. 2001). Also, like *Lr34*, *Lr46* is believed to confer resistance to all pathogen races (Martinez et al. 2001); although contradictory evidence has shown the gene to be ineffective against Indian leaf rust races (Agarwal and Saini 2009). In 'Pavon76', *Lr46* works in an additive fashion with at least two other APR genes, independent of known major genes *Lr10* and *Lr13* (Singh et al. 1998). William et al. (2003) showed that *Lr46* limits disease by diminishing infection frequency and reducing the size of uredinia. In addition, *Lr46* also acts to increase the latent period prior to infection. This is similar to the defense response of *Lr34* (William et al. 2003), which prevents visible symptoms of leaf rust infection until six weeks post-infection (Krattinger et al. 2009). However, the effect of *Lr46* alone was not shown to provide adequate protection against leaf rust, since disease ratings of up to 60% severity were recorded (Singh et al. 1998). This is in agreement with other studies that have described *Lr46* as being less effective than *Lr34* (Martinez et al. 2001, Lillemo et al. 2008, Hiebert et al. 2010, Herrera-Foessel et al. 2011,Lagudah 2011).

Another similarity is that *Lr46* also appears to be pleiotropic or tightly linked to additional genes providing partial disease resistance to powdery mildew (*Pm39*) (Lillemo et al. 2008) and stripe rust (*Yr29*) (Bariana et al. 2007, William et al. 2003). In addition, *Lr46* is also associated with the LTN phenotype, and has been given the gene designation *Ltn2* (Rosewarne et al. 2006). To date, attempts to knock-out the function of one of these genes has resulted in a loss of expression of all associated traits, suggesting all traits are under pleiotropic control (Lagudah et al. 2007). In some genetic backgrounds, the severity of LTN associated with *Lr46* appears to be less than the severity of LTN associated with *Lr34* (Rosewarne et al. 2006). This fits with the current hypothesis that *Lr34* appears to be more effective when compared to *Lr46* (Lillemo et al. 2008).

Several studies have performed genetic mapping experiments to localize *Lr46* in the wheat genome. William et al. (2006) used bulk segregant analysis (BSA), and partial linkage mapping, to identify several markers that may be useful for marker assisted selection for *Lr46* (Table 3). In the cross 'Avocet S' x 'Pavon76', two SSR markers, *Xgwm140,* and *Xgwm259*, located 13.7 cM

apart on chromosome 1BL were associated with *Lr46.* An additional SSR marker *Xwmc44* was identified 3.5 cM from the *Lr46* locus. The authors suggest that using this combination of three flanking markers may be helpful as a selection tool for *Lr46*/*Yr29* in molecular breeding programs. In the cross Avocet/Saar, molecular markers *Xwmc719* and *Xhbe248* were found to be more tightly flanking to the locus with a distance of 6.8 cM when powdery mildew disease association was used for the analysis (Lillemo et al. 2008). To date, attempts to mine the *Lr46* genomic region for the presence of ABC transporter sequences similar to the one encoded by *Lr34* have not been successful, which could indicate that *Lr46* confers resistance by a different mechanism (Lagudah 2011). The distribution of *Lr46* among Canadian wheat cultivars is unknown.

2.6.2.3 *Lr67/Yr46*

Hiebert et al. (2010) confirmed the presence of a third APR gene in wheat, and proposed the gene designation *Lr67*. The frequency of *Lr67* in germplasm worldwide is unknown (Singh et al. 2010).The gene has been localized to the centromeric region of chromosome 4DL by examining the crosses: Thatcher/RL6077 and RL6058/RL6077. Initially, RL6077 was proposed by Dyck et al. (1994) to carry *Lr34* resulting from a chromosomal translocation. Evidence for the translocation was due to the presence of quadrivalents in the pollen mother cells of the RL6077/RL6058 derived progeny. However, in later studies, Lagudah et al. (2009) determined that RL6077 does not carry *Lr34*, and concluded that the APR phenotype is likely due to a previously unidentified gene. Further confirmation was provided when the complete *Lr34* ABC transporter gene sequence was compared to the sequence from RL6077, which showed a haplotype associated with the susceptible *Lr34* allele (Herrera-Foessel et al. 2011).

Lr67 shares many characteristics with *Lr34* and *Lr46*. The gene is pleiotropic or linked to a gene (*Yr46*) conferring APR to stripe rust (Herrera-Foessel et al. 2011). Also, *Lr67* is associated with LTN (Hiebert et al. 2010, Herrera-Foessel et al. 2011), but to date, no gene name has been assigned to that phenotype. It is not yet known if *Lr67* confers resistance to powdery mildew (Hiebert et al. 2010). Two studies have shown that lines carrying *Lr34* express stronger leaf rust resistance when compared to lines carrying *Lr67* (Hiebert et al. 2010, Herrera-Foessel et al.2011).

Table 3: Molecular markers for APR genes *Lr34, Lr46* and *Lr67*.

Gene	Marker	Description	Reference
Lr34	*csLV34*	Wide diagnostic ability among international germplasm (Kolmer et al. 2008, Ellis et al. 2007). Not diagnostic in some lines (AC Domain)	(Lagudah et al. 2006)
	csLVMS1	0.13 cM from locus	(Spielmeyer et al. 2008)
	CaIND11	Co-dominant, robust, and gene specific	(Dakouri et al. 2010)
	CaISBP1	Insertional based-polymorphism, tightly linked	(Dakouri et al. 2010)
	Lr34	Distinguishes the rare mutation in cultivar 'Jagger'	(Cao et al. 2010)
Lr46	*Xgwm140*	0.3 recombination frequency	(Kuchel et al. 2007)
	Xgwm259	Flank locus	William et al. 2006)
	Xwmc44	3.5 cM from locus	(Suenega et al. 2003)
	Xwmc367	Flank locus	(William et al. 2006)
	Xwmc719	Flank locus	(Lillemo et al. 2008)
	Xhbe248	Flank locus	(Lillemo et al. 2008)
	XSTS1BL17	5.6 cM from locus	(Mateo Hernandez et al. 2006)
	XSTS1BL18	9.3 cM from locus	(Mateo Hernandez et al. 2006)
	XSTS1BL9	No recombination detected	(Mateo Hernandez et al. 2006)
	Lr46	Diagnostic KASP marker	(Gina Brown-Guedira, unpublished data)
Lr67	*Xcfd71, Xbarc98, Xcfd23, Xwmc457*	No recombination detected	(Hiebert et al. 2010) (Herrera-Foessel et al 2011)
	Cfd71, Cfd23	SSR marker 5.2 cM from locus useful in many backgrounds	(Hiebert et al. 2010).
	Xgwm192 *Xgwm165*	0.4 cM distal to locus	(Herrera-Foessel et al. 2011)

However, both studies found that *Lr67/Yr46* confers a similar resistance to stripe rust when compared to *Lr34/Yr18*.The LTN associated with *Lr67* is of similar severity to the LTN associated with *Lr34/Ltn1* (Hiebert et al. 2010), but is much more severe than the LTN associated with *Lr46/Ltn2* (Herrera-Foessel 2011). Interestingly, in both studies *Lr46* expressed the weakest level of leaf and stripe rust resistance of the three APR genes.

Two SSR markers, X*cfd71* and X*cfd23* (Table 3) have been suggested to be useful when selecting for *Lr67* in most genetic backgrounds, but additional work to develop more closely linked, high throughput and robust DNA markers will be required (Hiebert et al. 2010). Herrera-Foessel et al. (2011) identified two additional markers *Xgwm192* and *Xgwm165* (Table 3), both mapping 0.4 cM distal to the *Lr67* locus (Herrera-Foessel et al. 2011). Further work will be required to determine if these markers will be useful in marker assisted breeding programs (Herrera-Foessel et al. 2011).

2.7 Additive Interaction between APR Genes and Marker Assisted Selection

Molecular markers are of increasing importance to wheat breeders as a tool for disease resistance breeding. First they allow breeders to monitor and select for desirable resistance genes in segregating populations, and second, they allow for germplasm screening and subsequent identification of resistance genes in lines of unknown origin (Vida et al. 2009). To be most beneficial to breeders, markers should be co-dominant, which allows for homozygous resistant lines to be selected at all stages of the breeding program (Ellis et al. 2007). Furthermore, the markers must be tightly linked to the gene in question with limited ascertainment bias in a wide range of genetic backgrounds, amendable to high throughput platforms, and cost-effective (Ellis et al. 2007).

Molecular markers can be helpful to breeders when selection is otherwise impossible. This holds especially true when selecting for APR where disease ratings can be unreliable due to the large effect of environment and growth stage on disease development (Mateos-Hernandez et al. 2006). This problem is compounded when there is the potential for epistatic interaction between major seedling genes over APR genes (Lagudah 2011). Molecular markers can help breeders overcome these problems and allow the tracking of resistance genes during the development of cultivars with superior disease resistance characteristics. The molecular markers available for the three multi-pathogen APR genes in wheat listed in Table 3 could provide a useful tool for stacking these resistance genes in adapted breeding lines.

There is some evidence that suggests APR genes can be combined to provide near immune responses to leaf rust (Singh et al. 2000a). Similarly, cultivars with stacked APR genes are predicted to be more stable across environments (Singh et al. 2010). However, phenotypic evaluation of APR is often difficult due to interactions with genetic background, interactions with major genes, or with the environment (Lagudah 2011). Some studies have shown that the additive effects of *Lr34* and *Lr46* does not fit a traditional additive gene model, since the resistance provided by *Lr34* alone was similar to the resistance provided by *Lr34+Lr46* (Lillemo et al. 2008, Herrera-Foessel et al. 2009). The non-additive response could have been a result of low of disease pressure during screening, or because the two genes share mechanistic similarity in their defense responses (Martinez et al. 2001, Lillemo et al. 2008). These studies also showed that the disease resistance conditioned by *Lr34* was stronger than that of *Lr46* for all three diseases, and that the correlation was high between stripe rust and leaf rust disease severities (Lillemo et al. 2008). In a similar study, Dyck et al. (1994) examined the combined effect of *Lr34* and *Lr67* and found little phenotypic difference when compared to the effects of *Lr34* alone. To date, only a limited number of studies have examined the additive effects in an *Lr34 +Lr46 +Lr67* stacked cultivar. Taken together, these studies provide insufficient evidence for additive interaction between the known APR genes and highlight an important area for future research.

Future work should focus on identifying additional APR genes and testing their effectiveness when combined, as well as characterizing their interaction with major seedling genes. One benefit of combining resistance genes is that should one resistance gene fail due to a shift in the pathogen population, durability would be maintained due to the presence of the other genes (Lagudah 2011). Some APR genes, such as *Lr34*, have been described to work synergistically with other major resistance genes to provide an enhanced level of resistance (German and Kolmer 1992). Therefore, there is a significant need to identify and clone more APR genes (Ellis et al 2007).

3.0 MATERIALS AND METHODS

3.1 Plant Materials

A mapping population of 185 doubled-haploid (DH) lines derived from the cross RL4452/AC Domain was developed previously at Agriculture and Agri-Food Canada (AAFC), seeds of which were kindly provided by Dr. Gavin Humphreys for use in this study. RL4452 (Glenlea * 6/Kitt) is a confirmed carrier of *Lr34* and expresses strong APR in the field (McCallum et al. 2012a). AC Domain (ND499/RL4137//ND585) expresses comparable APR in the field and could carry the APR gene *Lr46* (Brent McCallum, unpublished data). AC Domain also carries the partially effective seedling gene *Lr16* and the defeated seedling gene *Lr10* (McCartney et al. 2005b). Randomized checks were included at all locations, which included the parents of the initial cross, the susceptible check Thatcher, and two near isogenic lines (NILs) of Thatcher : Thatcher-*Lr16* and Thatcher-*Lr34*.

Additional molecular analyses were performed using a population of a diverse set of hexaploid and tetraploid cultivars (herein referred to as the diversity panel; See Appendix 5). This population was also screened with available molecular markers for *Lr34* and *Lr46* (See Appendix 5). In addition, WPCH-09 (Colin Hiebert, personal communication), which is a confirmed carrier of *Lr46,* was used as a positive control for genotyping at that locus.

3.2 Experimental Design

During 2011-2012, field disease trials were grown at three locations in western Canada. In both years, leaf rust nurseries were planted at Saskatoon, SK and Portage, MB, and a stripe rust nursery was established at Lethbridge, AB. At Saskatoon, experimental lines and checks were seeded in 1 m single rows with 30 cm row spacing. The trial was set-up as an alpha lattice plot design with 48 incomplete blocks nested within a randomized complete block design (RCBD) with 3 replications. The Portage leaf rust nursery was set up in an RCBD with 3 replications. Because of seed availability, the Lethbridge stripe rust nursery was planted only as a single replication in 2011, but as an RCBD with 3 replications in 2012.

At Saskatoon and Portage, spreader rows consisting of highly rust susceptible lines were seeded two weeks prior to the trial seeding dates to facilitate the spread of the rust epidemic. A representative collection of *P. triticina* isolates gathered in western Canadian fields during 2010 was used as inoculum for 2011. Likewise field isolates collected in 2011 were used for

inoculation of the 2012 leaf rust nurseries. The urediniospores were desiccated after collection and stored in vials at -80ºC. Before use, urediniospores were heat shocked by placing the vial in a water bath at 45ºC for 5 minutes. Dried urediniospores were then suspended in light mineral oil (Bayol 55, Imperial Oil, Toronto, ON, Canada). The urediniospore suspension was used to inoculate the spreader rows in the field. Two leaf rust inoculations were performed; the first inoculation was performed when the spreader rows were at the 3 leaf stage and the second inoculation approximately 2-3 days later. After inoculations, spreader rows were covered with tarps for 24 hours to keep humidity high, ensuring a long enough leaf wetness period to promote infection. The nurseries were irrigated periodically throughout the summer. At Lethbridge, where local stripe rust epidemics occur on an annual basis, the disease nursery relied solely on natural stripe rust infection.

3.3 Phenotypic Analysis

Rust ratings were taken using a modified Cobb scale of disease severity (DS) (Peterson et al. 1948). This rating scale describes the actual percentage of the flag leaf covered with rust uredinia in increments of 0, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%. The first rating was taken when the susceptible check Thatcher had reached a DS of 50%. At Saskatoon and Portage, host response to infection was recorded at the time of the first rating according to the guidelines presented in Roelfs et al. (1992), with lines classified as: R = resistant with necrotic and chlorotic strips with no sporulation, MR = moderately resistant with necrosis and chlorosis with some sporulation, M = intermediate with necrosis, chlorosis and sporulation, MS = moderately susceptible with chlorotic areas, minimal necrosis and abundant sporulation , and S = susceptible, with or without chlorotic areas and abundant sporulation. At Saskatoon, subsequent ratings were taken at 4-5 day intervals until flag leaves had begun to senesce.

Leaf tip necrosis (LTN) was scored only at the Saskatoon environments in both 2011 and 2012. For scoring, an ordinal scale of 0 to 5 was used to quantify severity, where 0 = no visible LTN, 5= extreme LTN.

3.4 Molecular Analysis

DNA was extracted and pooled from 3 representative plants for each experimental line, parents and checks. Tissue was taken from week-old seedlings and was subsequently lyophilized for 24 hours prior to beginning DNA extraction. Plant genomic DNA extraction was performed

using a modified CTAB method (Hoisington et al. 1994). Genomic DNA quantitation was performed using PicoGreen (Invitrogen) fluorescence detection, and all DNA samples were diluted to 50 ng/µl. For Picogreen analysis, a standard curve was generated using Lamda DNA of known concentrations.

Polymerase Chain Reactions (PCR) reactions were performed according to McCartney et al. (2005b) with certain laboratory-specific modifications. PCR reactions were performed in either 96, or 384 well plates with total reaction volumes of 25 µl or 15 µl respectively. Each reaction contained 50 ng of genomic DNA, 0.5 U of Genscript Taq DNA polymerase (Genscript), 1X Genscript PCR buffer (Genscript), 1.5mM $MgCl_2$, 200µM of each dNTP, 200 µM of reverse primer, 20 µM of forward primer modified to contain 19 bp M13 tail CACGACGTTGTAAA (Scheulke 2000), and 180 µM of 6-FAM/HEX/NED-labeled M13 primer. The general PCR program consisted of initial denaturation at 94ºC for 3 min, followed by 12 cycles of 94ºC for 45 s, 56ºC (or 62ºC) for 55 s (Decreasing 2ºC every 3 cycles) 72 ºC for 50 s; followed by 33 cycles of 94ºC for 30 s, 51ºC for 45 s, 72ºC for 45 s; with a final extension step of 72ºC for 15 min. Polymorphisms were resolved using capillary electrophoresis on an ABI3130 (Applied Biosystems, Foster City, California) and peaks were manually scored for size using a known size standard. In some cases, polymorphisms with a size difference greater than 20 bp were scored on agarose gels. A 1 Kb+ DNA ladder was included on agarose gels to estimate the size of the amplified fragment.

KASP analysis was performed according to the guidelines in the KBIOscience KASP version 4.0 SNP Genotyping manual (www.kbioscience.co.uk). Reactions were performed in 384 well plates, with a final reaction volume of 8 µl. Reaction mixtures contained 1X general reaction mix (www.kbioscience.co.uk), 50 ng of template DNA, 0.165 µM Hex forward primer, 0.165 µM FAM forward primer and 0.412µM universal reverse primer. Thermocycling and plate reading was performed on a Bio-Rad C1000 thermocycler (Bio-Rad Laboratories Ltd). The PCR program consisted of 94ºC for 15 min, followed by 10 cycles of 94ºC for 20 s, 65-57ºC (dropping 0.8ºC per cycle) for 60 s , then 26 cycles of: 94ºC for 20 s, 57ºC for 60 s, with a final fluorescence plate reading taken at 10ºC.

Single stand conformational polymorphism (SSCP) analysis was performed according to the procedures outlined in Wiebe et al. (2010). Briefly, 4 µl of PCR product was added to 20 µl of loading buffer that consisted of 95% formamide, 0.05% bromophenol blue and 0.05% xylene

cyanol. To induce single strand folding, the sample mixture was heated at 94ºC for 5 min, then immediately removed and placed on ice. Fragments were resolved on 0.6X MDE gels (Lonza, Rockland, ME, USA) , with 0.6X TBE run buffer, using the Bio-Rad Sequi-Gen GT electrophoresis system. Fragments were visualized by silver staining as described in Bassam and Gresshoff (2007).

3.5 Map Construction and QTL Analysis

Using the *Lr34* diagnostic primer *caISBP1* (Dakouri et al. 2010), the RL4452/AC Domain population was split into *Lr34* carriers and *Lr34*-non carriers. The latter sub-group consisted of 93 lines from which DNA was used for Infinium iSelect 9K SNP genotyping. Out of nearly 9000 SNPs, approximately 1900 were polymorphic between the parents, which were scored in the DH population and used for linkage map construction within Joinmap 4.0 (Van Ooijen 2006). Any SNP markers with call frequencies of less than 75%, and all markers showing significant segregation distortion were removed from linkage mapping experiments. A minimum logarithm of odds (LOD) score of 4.0 was used as a starting point for assigning markers to linkage groups, but because marker coverage was high, it was necessary to select a LOD score of up to 10.0 for some linkage groups. All linkage maps were constructed using the maximum likelihood (ML) mapping algorithm. The program GMAP (Wu and Watanabe 2005) was used to align all iSelect 9K probe sequences against the Chinese Spring survey sequence developed by the International Wheat Genome Sequencing Consortium using a cut-off value of 95% sequence identity and 80% sequence coverage to return a list of putative chromosome assignments for each SNP marker.

QTL analysis was performed separately for each environment using MapQTL 6.0 software (Van Ooijen 2009). Phenotypic traits analyzed included leaf rust DS Saskatoon (LRS) and Portage (LRP), stripe rust DS Lethbridge (YRL), coefficient of infection at Saskatoon (CIS) and Portage (CIP), area under the disease progress curve Saskatoon (AUDPC) and leaf tip necrosis Saskatoon (LTN). Least-square means (LSMEANS) derived from the SAS analysis (Section 3.7) was used for all QTL analysis. Simple interval mapping was first performed to identify genome regions from which cofactors were selected at 5 cM intervals, and validated through regression-based backwards elimination at a significance level of $P<0.020$. From this analysis a final set of cofactors was selected for use in multiple QTL mapping (MQM). Final linkage maps illustrating QTL were prepared using MapChart software (Voorips 2002).

3.6 Primer Design

For those SNP markers associated with important QTL, KASP and SSCP markers were developed for testing in the complete DH population. For KASP marker primer design, probe sequences were aligned using Basic Local Alignment Search Tool (BLAST) against the Chinese Spring survey sequence, and primers were designed to only target the sequence from the chromosome on which the QTL were identified. Using the web-based software Primer3 (Rozen and Skaletzky 2000), primer sequences were identified that were suitable for conversion from the iSelect probe sequence, to PCR-based KASP and SSCP markers. Candidate markers were tested by genotyping the *Lr34* non-carriers and comparing the generated scores against the original iSelect 9K derived genotypic scores. Only those markers with a near-perfect association between the two assays were used for further analysis. Successful markers were used to screen the *Lr34* carrying lines so that statistical analysis could be performed on the overall dataset.

3.7 Statistical Analysis

The coefficient of infection (CI) was calculated for Saskatoon and Portage leaf rust data according to the procedures outlined in Roelfs et al. (1992) by multiplying the percent disease severity by the corresponding coefficient of host response, where immune = 0.0, R = 0.2, MR=0.4, M=0.6, MS = 0.8, S = 1.0.

Area under the disease progress curve (AUDPC) was calculated using the multiple ratings taken at the Saskatoon leaf rust nurseries according to the following formula:

$$\sum_{i=1}^{n-1} \left(\frac{x_{i+1}+x_i}{2} \right) (t_{i+1} - t_i)$$

Where:

x_i is disease severity at the i^{th} observation
t_i is the time of the first disease rating
n is the total number of scoring dates in the trial.

All phenotypic data collected were analyzed separately for each environment using the MIXED procedure of SAS 9.2 statistical software (SAS Institute Inc., Cary, NC, USA). The genotype (i.e. experimental line) effect was portioned as a random factor to include the nested effect of relevant known resistance genes and important loci identified in QTL analysis, hereafter referred to as the genotype(loci) effect. The Saskatoon nursery was analyzed as an alpha-lattice considering incomplete blocks nested within replications [replication(block)], replication, and

loci nested within genotype [genotype(loci)] as random effects. The Portage leaf rust data was analyzed as an RCBD considering replication, and loci nested inside genotype [genotype(loci)] as random effects. The 2011 un-replicated Lethbridge stripe rust data was analyzed as a single replication with loci nested within genotype [genotype(loci)] considered as a random effect, and the 2012 replicated stripe rust data was analyzed the same as the Portage data listed above. The fixed effects for the above models considered all possible allelic combinations between the known segregating genes *Lr34, Lr46, Lr16* and important loci associated with newly identified QTL.

In cases of significant ANOVA F-tests for fixed effects, comparison of treatment means was performed using a Fisher's protected least significant difference (LSD) at a significance threshold of $P<0.05$. In the case of a significant interaction between two or more fixed effects, only the data for the highest order interaction is presented. Pearson's correlation coefficients were calculated using the CORR procedure of SAS for all traits.

3.8 Growth Chamber Seedling Tests

Seedling leaf and stripe rust screening was carried out in separate experiments to determine if seedling resistance genes may be segregating in the population. The lines evaluated included: AC Domain and RL4452, the susceptible checks Avocet (stripe rust) and Thatcher (leaf rust), and Thatcher-*Lr16* (leaf rust). Plants were grown in 32 cell (8 by 4) root trainers in an RCBD design with 3 replications. One seed was planted for each cell, and between 4 and 8 plants were evaluated per line, for each replication. A mixture of *P. striiformis* races collected during the 2011 field season at Lethbridge was used for all stripe rust seedling testing. The same mixture of *P. triticina* races used for 2012 field testing at Saskatoon and Portage was used for leaf rust seedling testing. All rust urediniospores were heat shocked prior to inoculum preparation by placing containing vials in a water bath set to 45ºC for 5 minutes. Inoculum was prepared so that 0.01g of spores was suspended in 300 µL of Bayol oil (Imperial Oil, Toronto, ON, Canada). Ten day old seedlings were inoculated using a pneumatic sprayer applying inoculum to plants as evenly as possible. After inoculation, the oil was left to evaporate off the leaves for at least one hour. The plants were placed in a dark humidity chamber for 48 hours at 10ºC (stripe rust) or 24 hours at 18º C (leaf rust) , then were placed in separate growth chambers set at 15ºC day /10ºC night (stripe rust) and 22ºC day /18ºC night (leaf rust) temperatures, with 16 hour photoperiods. Stripe rust ratings were recorded at two day intervals beginning at 14

days post inoculation, following the McNeal 1-9 stripe rust rating scale (Roelfs et al. 1992), and results were analyzed statistically using SAS v.9.2 Stripe rust seedling data was analyzed as an RCBD considering replication as a random effect and genotype as a fixed effect. Leaf rust infection types (IT) were recorded 12 days after inoculation according to the 0-4 rating scale in Roelfs et al (1992). For leaf rust multiple ITs were often noted complicating statistical analysis, therefore raw leaf rust seedling data was combined across reps, and disease ratings were presented in order of the most predominant IT.

4.0 RESULTS

4.1 Genetic Characterization of *Lr34*

The presence or absence of *Lr34* in the DH lines was determined using the gene specific primers *caISBP1* (Figure 1) and *caIND11* (Dakouri et al. 2010, McCallum et al. 2012a). Results confirmed *Lr34* was segregating (Figure 1), and these data were used to classify lines into *Lr34* (+) and *Lr34* (-) genotypic groups for subsequent analysis. These results confirmed that RL4452 carries *Lr34*, and reaffirmed that AC Domain is a non-carrier (Figure 1) . Preliminary analysis revealed that *Lr34* had a large effect on leaf and stripe rust resistance in the DH population (See section 4.2) and in the majority of cases, the presence of *Lr34* masked the potential expression of other resistance loci.

Figure 1: An agarose gel showing the banding pattern of the *Lr34* diagnostic primer *caISBP1* (Dakouri et al. 2010) in the parents and a subset of the RL4452/AC Domain mapping population. The larger fragment (top band, 509 bp) is associated with the *Lr34* (+) allele. The smaller fragment (bottom band, 391 bp) is associated with the *Lr34(-)* allele.

4.2 Field Disease Screening

The ANOVA for each testing environment is presented in Appendix 1, and the LSmeans for all experimental lines in all test environments is presented in Appendix 2. Over the two years of field testing, all disease nurseries had good levels of rust infection. The DH lines of the mapping population expressed a wide range in disease severity (DS) (Figure 2). Some lines showed transgressive segregation and were more resistant than the parents, while other lines were as susceptible as Thatcher (Table 4). Because the data showed a genotype by environment interaction (data not shown), results are presented for each environment separately. The resistance conferred by *Lr34* was found to be substantial in all environments, for all traits. As such, the distributions were separated into *Lr34* (+) and *Lr34* (-) subgroups to better visualize the distribution of disease reactions among the two genotypic classes (Figure 2). Interestingly, the distribution of lines in the *Lr34* (-) subgroup showed that some lines still expressed APR to both

diseases while other lines were almost completely susceptible (Figure 2). This suggests that there are additional genetic factors segregating in the population that are independent of *Lr34*.

Table 4: Average percent disease severity (DS) recorded on checks during 2011-2012 field testing in rust nurseries at Saskatoon, Portage, and Lethbridge. The mid-parental values (MPV) were calculated from the parental rust DS scores. Also shown are the overall DH population means for each environment, along with mean disease severities in the *Lr34*+ and *Lr34*- subgroups.

	Leaf Rust				**Stripe Rust**	
	Saskatoon		Portage		Lethbridge	
Name	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
Thatcher	56.7	86.7	62.5	80.0	85.0	91.7
Thatcher-*Lr16*	50.0	76.7	53.3	-[a]	85.0	93.3
Thatcher-*Lr34*	13.3	21.7	7.5	30.0	20.0	25.0
AC Domain	15.0	31.7	22.5	40.0	65.0	26.7
RL4452	10.0	11.6	11.7	-	0	11.7
MPV	12.5	21.7	17.1	-	32.5	19.2
Mean DS *Lr34*(+)	5.1	11.5	9.3	16.2	10.0	20.9
Mean DS *Lr34* (-)	21.1	27.7	26.7	61.3	37.9	40.5
Population Mean	13.9	20.1	18.9	41.0	25.0	31.5
LSD *P* >0.05	9.8	15.4	18.3	18.7	x[b]	13.2

[a] "– " indicates missing data.

[b]x=no LSD could be estimated as data was collected from a single replicate.

4.2.1 Saskatoon

Statistical results showed there were significant differences ($P<0.001$) in disease reaction between lines in both years at Saskatoon (Appendix 1). Thatcher and Thatcher-*Lr16* were rated as highly susceptible at both environments, with both lines scoring disease severities greater than 50% (Table 4). In contrast, Thatcher-*Lr34*, AC Domain, and RL4452 all had DS of ≤ 15% in 2011 (Table 4).

In 2011, the mean DS in the DH population at Saskatoon was 13.9% (Table 4). In comparison, the mean DS in the *Lr34* carriers was 5 % (Table 4). The *Lr34* non-carriers expressed severities between 0-60% (Figure 2), and averaged 21.1% DS (Table 4). Correlation analysis showed that DS was highly correlated with CIS (r=0.970), and

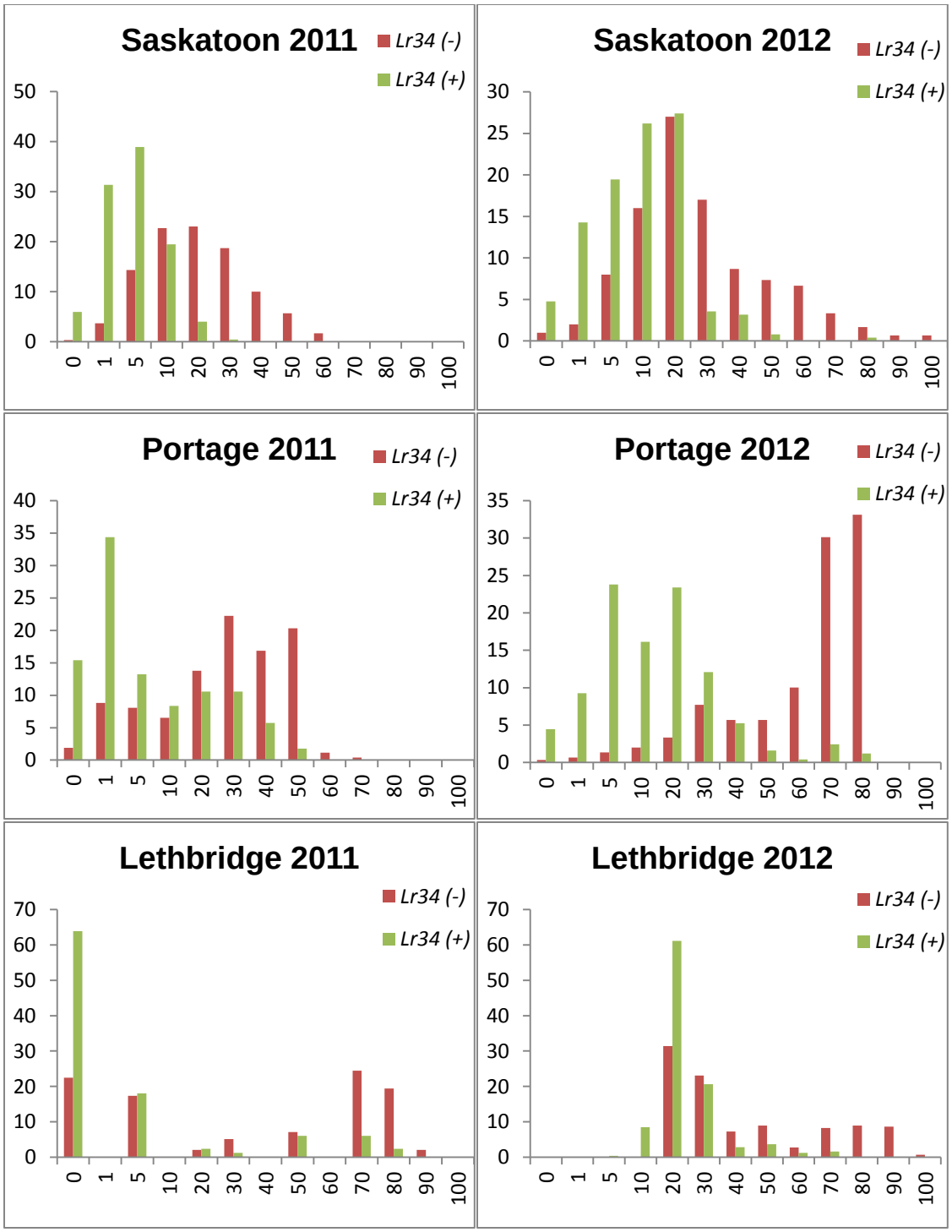

Figure 2: Proportional histograms displaying raw disease severities for field leaf rust nurseries at Saskatoon and Portage, and the stripe rust nursery at Lethbridge during 2011-2012. The Y-axis shows the proportion of experimental lines while the X-axis shows percent disease severity (DS). Experimental lines are separated by the presence or absence of *Lr34* to better visualize the disease reactions in each sub-group.

AUDPC (r=0.985), illustrating the usefulness of such ratings in relation to the quantification of disease severities (Table 5). In 2012, the mean DS in the DH population at Saskatoon was 20.1%, which was higher than the previous year (Table 4). The largest proportion of lines carrying *Lr34* scored between 10-20% DS, and no line from this sub-group exceeded 50 % DS (Figure 2). In contrast, DS in *Lr34* non-carriers spanned a wider range than was observed in 2011, with values ranging from 0 – 100 % DS (Figure 2). The average DS expressed in the *Lr34* non-carriers was 27.7%, which was also higher than the previous year. In 2012, Saskatoon DS was strongly correlated with AUDPC (r=0.971) and CIS (r=0.980, Table 5). The testing environments at Saskatoon were also highly correlated (r=0.834, Table 5), indicative that field results were consistent between both years of testing.

4.2.2. Portage

Analysis of the Portage data indicated the differences between lines were significant (P<0.001) for each year (Appendix 1). In 2011, the Portage disease nursery had some challenges due to early season flooding, but despite this, leaf rust infection was high. However, some plots were lost due to water stress and, as a result the variation at Portage was greater than was observed at Saskatoon. In both years, Thatcher and Thatcher-*Lr16* were susceptible, and expressed DS between 60-80% (Table 4). Unfortunately, data from Thatcher-*Lr16* and RL4452 were lost at Portage in 2012. In contrast to the highly susceptible checks, AC Domain expressed APR that ranged between 20 – 40% DS. In 2011, RL4452 was very resistant scoring only 11.6% DS.

Because only one disease rating was taken, AUDPC was not calculated for the Portage environments. The distribution of disease reactions showed that *Lr34* was still the most important genetic factor associated with leaf rust resistance (Figure 2). In 2011, the average DS in the *Lr34+* subgroup was 9.3% (Table 4). Similar to Saskatoon environments, the *Lr34* non-carriers expressed a wider range of DS, and averaged 26.7% DS (Figure 2). In 2011, Portage DS was highly correlated with CIP (r=0.909, Table 5).

Disease pressure at Portage during the 2012 field season was the highest of all testing environments (mean population DS = 41%, Table 4). The distribution of lines showed that *Lr34* had the strongest effect since the largest proportion of lines in the *Lr34+* subgroup scored between 5-20% DS (Figure 2). Interestingly, the largest proportion of lines in *Lr34* non-carriers were susceptible (DS>65 %, Figure 2) to leaf rust, which was not consistent with the other

testing environments. However, there were some *Lr34* non-carriers that expressed a high level of resistance. The 2012 Portage DS was strongly correlated with CIP (r = 0.976, Table 5). Similar to the Saskatoon environments, the two testing environments at Portage were positively correlated (*r*=0.751) across years (Table 5).

4.2.3. Lethbridge

Data for stripe rust reaction was collected over two years in naturally endemic nurseries at Lethbridge, Alberta. In 2011, data was only collected on a single replication, but in 2012, each of the checks and DH lines was replicated three times. The ANOVA for 2012 is presented in Appendix 1, which showed significant differences among lines ($P<0.001$). At Lethbridge, stripe rust disease pressure was high during the 2011 field season. Ample snow cover during the previous winter, and a relatively cool growing season allowed the pathogen to overwinter, which contributed to the severity of the epidemic (Harpinder Randhawa, Personal Communication). Thatcher and Thatcher-*Lr16* were rated as highly susceptible in both years, whereas Thatcher-*Lr34* showed intermediate disease severities (Table 4). AC Domain was scored as susceptible to stripe rust in 2011 (DS= 65%), but expressed APR to stripe rust in 2012 (DS = 26.7%)

Despite the high disease pressure in 2011, many lines in the mapping population expressed a high level of rust resistance (Figure 2, Appendix 2). The mean DS for stripe rust was 25% in the mapping population (Table 4). Most lines carrying *Lr34* were highly resistant to stripe rust (DS $\leq$ 5%), although it was noted that a few lines in the group were stripe rust susceptible (DS $\geq$65%, Figure 2). In contrast, the distribution of the *Lr34* non-carriers resembled a bimodal distribution, and a near equal proportion of lines showed either complete resistance ($\leq$5% DS), or complete susceptibility (DS >70%) to the disease (Figure 2), although a small proportion of lines expressed an intermediate level of resistance between these two groups. This could suggest segregation for a major resistance gene in the *Lr34* - subgroup.

In 2012, the mean population DS at Lethbridge was higher than the previous year (DS=32%, Table 4). In the *Lr34* non-carriers, DS ranged between 15 - 100% (Figure 2). In the *Lr34* carriers, the largest proportion of lines scored approximately 15 % DS, but ranged between 10-70% DS (Figure 2). The stripe rust DS ratings collected at Lethbridge were positively correlated (*r*=0.630) between years (Table 5).

4.3 Linkage and QTL Mapping Studies

4.3.1 Linkage Mapping and Genetic Analysis of Known Rust Resistance Genes

As noted in section 4.2, the analysis of field reaction to leaf and stripe rust revealed that *Lr34* had a large effect on disease resistance in the DH mapping population. In addition, separation of the DH lines into *Lr34+* and *Lr34-* classes revealed apparent segregation of additional leaf and stripe rust resistance genes that could be masked by *Lr34*. To identify additional QTL associated with these traits, a genetic map of the RL4452/AC Domain population was constructed by genotyping only the *Lr34* (-) portion of the population (93 lines).

McCartney et al. (2005b, 2006) had previously constructed a genetic linkage map in the RL4452/Domain mapping population consisting of 369 SSR markers across 27 linkage groups spanning a total map distance of 2793 cM. For the present study, genotyping was performed using a 9K Illumina iSelect SNP assay. Additional SSR markers were selected that were associated with known APR genes, and flanking QTL identified in QTL analysis (See Section 3.4). In total 1946 SNP markers were polymorphic between AC Domain and RL4452. After filtering for markers showing significant segregation distortion, 1884 high-quality, informative SNPs collected from the subset of 93 lines from the DH population were used for construction of a high density map. The final map consisted of 35 linkage groups spanning all 21 chromosomes of the wheat genome (Appendix 3). The total length of the high density genetic map was 3117 cM.

Given that AC Domain is not a carrier of *Lr34*, but expressed APR in the field (Table 4), it is possible that AC Domain may be carrying alternative, known APR genes. One hypothesis is that AC Domain may be a carrier of *Lr46* (B. McCallum, personal communication). To test this, a KASP marker linked to *Lr46* (Gina Brown-Guedira, unpublished data) was evaluated and was found to be polymorphic in the parents and mapping population (Figure 3). The *Lr46* (+) check WPCH-01 (Colin Hiebert, Personal Communication) was assayed with the parents and DH population, and revealed the population was segregating for *Lr46* (Figure 3). Interestingly, RL4452 showed the same allele as WPCH-09, suggesting this line, not AC Domain was contributing the *Lr46* (+) allele to the mapping population. The *Lr46* KASP marker was localized to chromosome 1AL (Figure 4), consistent with the reported location of *Lr46* (Rosewarne et al. 2006, William et al. 2006). Additionally, known *Lr46* flanking SSR markers

Xbarc80, *Xwmc44* and *Xgwm140* (Suenega et al. 2003, Rosewarne et al. 2006) were also segregating and these mapped within 13 cM of the KASP marker (Figure 4, Appendix 3). AC Domain is also a known carrier of *Lr16* (McCartney et al. 2005a,b) and is linked (<1 cM) to the SSR marker *Xwmc764*. As reported previously in this population (McCartney et al. 2005a,b), *Xwmc764* localized to the very distal end of chromosome 2BS (Figure 4, Appendix 3).

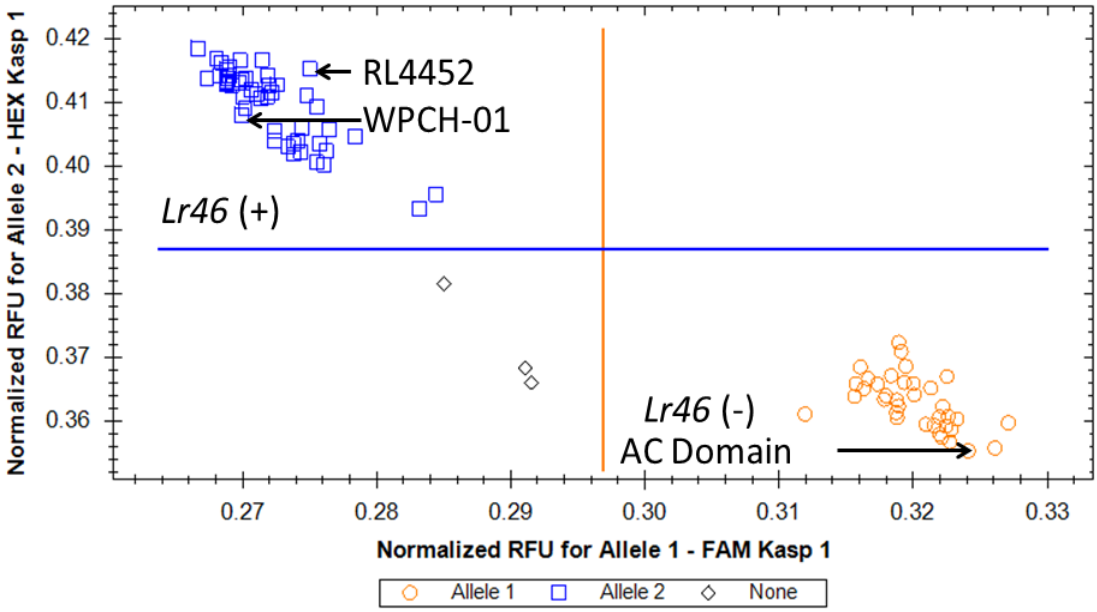

Figure 3: Scoring of the *Lr46* KASP marker on the parents and a subset of lines in the DH mapping population. Check cultivars are shown with an arrow. Lines carrying the *Lr46*+ marker allele separated into the upper left quadrant. These included RL4452, and the known *Lr46*+ check WPCH-01. Lines not carrying the *Lr46* + marker allele separated into the bottom right quadrant which included AC Domain. Lines in the bottom left quadrant are negative controls which contained no sample DNA.

Table 5: Pearson correlation coefficients calculated for phenotypic data collected during field testing in 2011-2012. All coefficients presented were significant at $P<0.001$.

	Saskatoon 2011	Saskatoon 2012	Portage 2011	Portage 2012	Lethbridge 2011	Lethbridge 2012	AUDPC 2011	AUDPC 2012	CIS 2011	CIS 2012	CIP 2011	CIP 2012	LTN 2011
Saskatoon 2012	0.834												
Portage 2011	0.753	0.664											
Portage 2012	0.731	0.586	0.751										
Lethbridge 2011	0.560	0.425	0.500	0.521									
Lethbridge 2012	0.591	0.514	0.471	0.512	0.630								
AUDPC 2011	0.985	0.837	0.740	0.722	0.544	0.567							
AUDPC 2012	0.855	0.971	0.682	0.635	0.447	0.518	0.867						
CIS 2011	0.970	0.820	0.694	0.652	0.530	0.568	0.960	0.832					
CIS 2012	0.816	0.980	0.632	0.544	0.419	0.492	0.826	0.954	0.808				
CIP 2011	0.674	0.571	0.909	0.659	0.435	0.417	0.669	0.590	0.633	0.550			
CIP 2012	0.763	0.622	0.749	0.976	0.547	0.545	0.762	0.664	0.693	0.588	0.673		
LTN 2011	-0.533	-0.473	-0.523	-0.551	-0.363	-0.411	-0.514	-0.479	-0.501	-0.441	-0.428	-0.549	
LTN 2012	-0.553	-0.632	-0.427	-0.347	-0.248	-0.354	-0.556	-0.623	-0.545	-0.627	-0.316	-0.354	0.281

4.3.1 QTL Mapping Results

Results from the QTL mapping analysis are presented in Table 6 and Figure 4. All QTL were named according to the Recommended Rules for Gene Symbolization in Wheat (McIntosh et al. 1998), where QTL were designated as *usw* (designator for Dr. Pozniak's laboratory, Crop Development Centre, University of Saskatchewan). At Saskatoon, two leaf rust QTL for DS (*QLrs.usw-2B1, QLrs.usw-2B2)* were identified, and both were derived from AC Domain. Of these, *QLrs.usw-2B1* reached a higher LOD score, explained a greater proportion of the phenotypic variation, and was the only QTL detected in both years (Table 6). *QLrs.usw-2B1* peaked at the co-segregating loci *wsnp_Ex_c16144_24583060* - *wsnp_Ra_rep_c117300_96881829,* and explained 25.3% (LOD 11.25) and 38.9% (LOD 13.64) of the total leaf rust variance in 2011 and 2012, respectively. The second QTL *QLrs.usw-2B2* was only detected in 2011, and mapped approximately 46 cM proximal to *QLrs.usw-2B1*. *QLrs.usw-2B2* peaked at the *Lr16* diagnostic SSR marker *Xwmc764* (LOD 3.76) and accounted for 7% of the total leaf rust variance in 2011. However, *QLrs.usw-2B2* was not detected in 2012. At Portage, two QTL (*QLrp.usw-2B1, QLrp.usw-4A*) were identified for leaf rust DS. *QLrp.usw-2B1* had the same peak, and spanned a similar marker interval to *QLrs.usw-2B1* (Table 6). In 2011, *QLrp.usw-2B1* explained 41.7 % (LOD 13.54) of the leaf rust variance, and was the only QTL detected during that year. In 2012, *QLrp.usw-2B1* explained 21.8% (LOD 6.67) of the leaf rust variance. In addition, a second QTL: *QLrp.usw-4A* was located on chromosome 4A that explained 12% (LOD 3.93) of the total leaf rust variance in 2012. The allele for resistance at *QLrp.usw-4A* was also derived from AC Domain.

At Lethbridge, three significant QTL (*QYr.usw-2B1, QYr.usw-4A, and QYr.usw-6B)* were identified for stripe rust in 2011-2012. *QYr.usw-2B1* spanned approximately the same interval as *QLrs.usw-2B1* and *QLrp.usw-2B1* and peaked at *wsnp_Ex_c16144_24583060* - *wsnp_Ra_rep_c117300_96881829,* except in 2011 when it peaked at the adjacent locus *Wsnp_Ex_rep_c66551_64836462* located approximately 13 cM distal to its 2012 peak (Table 6, Figure 4). In 2011, *QYr.usw-2B1* explained between 8.6-10.7% (LOD 3.29-3.65) of the total stripe rust variance across both years. *QYr.usw-6B* explained 18.2% (LOD 7.6), and 13.9% (LOD 4.63) of the stripe rust variance, while *QYr.usw-4A* explained 9.2% (LOD 3.51), and 11% (LOD 3.76) of the stripe rust variance in 2011 and 2012, respectively. The resistance alleles for

QYr.usw-2B1 and *QYr.usw-4A* were both derived from AC Domain, while the resistance allele for *QYr.usw-6B* was derived from RL4452.

Four QTL (*QCis.usw-2B1, QCis.usw-4A, QCis.usw-3B, and QCis.usw-1B)* were detected for CI at Saskatoon over the course of the study, but only *QCis.usw-2B1* was detected in both years. *QCis.usw-2B1* shared the same peak loci with *QLrs.usw-2B1*, *Q.Lrp.usw-2B1* and *QYr.usw-2B1* (2011 only), and explained 30.8% (LOD 11.5), and 36.3% (LOD 12.24) of the total CIS variance in 2011, and 2012, respectively. The second QTL identified in 2011 was *QCis.usw-4A*, which explained 8.6% (LOD 3.92) of the total CIS variance. In 2012, two additional minor QTL (*QCis.usw-3B, QCis.usw-1B)* were detected. *QCis.usw-3B* explained 7.1% (LOD 3.05) and *QCis.usw-1B* explained 7.1 % (LOD 3.03) of the of the total CIS variance. Notably, the resistance allele for *QCis.usw-1B* was derived from RL4452, and peaked at the *Lr46* associated SSR marker *Xwmc44*. These results were consistent with the molecular results described previously, which showed RL4452 was an *Lr46* carrier.

Only two QTL (*QCip.usw-2B1* and *QCip.usw-5B*) were identified for CI at Portage (CIP), with the latter only being detected in 2012. In both cases, the resistance allele was derived from AC Domain. The most important QTL, *QCip.usw-2B1,* was coincident with *QLrs.usw-2B1*, *QLrp.usw-2B1, QYr.usw-2B*, *QCis.usw-2B1,* and explained 23.95% (LOD 6.1), and 25% (LOD 8.31) of the total CIP variance in 2011 and 2012, respectively. In 2012, *QCip.usw-5B* explained 10.2% (LOD 3.81) of the total CIP variance. 5 QTL were identified for AUDPC over the course of the study. Of these, the most important QTL, *QAudpc.usw-2B1,* was coincident with *QLrs.usw-2B1*, *QLrp.usw-2B1, QYr.usw-2B*, *QCis.usw-2B1*, *QCip.usw-2B1* and was the only QTL identified in 2011 and 2012, where it explained 43.3% (LOD 13.30) and 42% (LOD 14.51) of the total AUDPC phenotypic variance, respectively. In 2011, a second QTL, *QAudpc.usw-2B2,* was located peaking at *Xwmc764* that explained 12.1% (LOD 4.68) of the AUDPC variance for that year. This QTL was coincident with the 2011 Saskatoon leaf rust QTL *QLrs.usw-2B2*. Also in 2011, *QAudpc.usw-4A* explained 10.1% (LOD 3.84) of the AUDPC variance. The peak locus for *QAudpc.usw-4A* was approximately 5 cM distal to the 2011 CIS QTL *QCis.usw-4A*. In 2012, *QAudpc.usw-3B* explained 8.6% (LOD 3.91) of the AUDPC variance, and was coincident with the QTL *QCis.usw-3B* identified in the same year. Finally, a minor QTL *QAudpc.usw-1B* was located that explained 7.3% (LOD 3.36) of the variance, coincident with the 2012 QTL *QCis.usw-1B*. With the exception of *QAudpc.usw-1B*, all resistance alleles for AUDPC QTL

were derived from AC Domain. *QLtn.usw-2B1* was the only significant QTL identified for LTN during 2011 and 2012, and accounted for 14.6% (LOD 4.47) and 19.5% (LOD 6.31), respectively. The positive allele associated with increased LTN severity was derived from AC Domain.

4.4 Conversion of Infinium Probe Sequence to PCR-Based Markers.

The *Q.usw-2B1 (QLrs.usw-2B1, QLrp.usw-2B1, QYr.usw-2B1, QCis.usw-2B1, QCip.usw-2B1, QAudpc.usw-2B1, QLtn.usw-2B1)* locus was strongly associated with all measured traits (Figure 4), but the QTL-associated SNP markers on the 9K chip needed to first be converted to single-plex assays for validation and for use in breeder selection programs. Using the source sequence from which 9K iSelect probes were originally developed, PCR primers were designed for two SNPs most associated with *Q.usw-2B1*, namely: *wsnp_Ra_rep_c117300_96881829* and *wsnp_Ex_c16144_24583060*. To develop these markers, iSelect probe sequences were aligned against the Chinese Spring survey sequence using BLAST, specifically targeting chromosome 2BS. Each iSelect probe sequence was matched to a specific contig from Chinese Spring, and within each contig, the target SNP for which the iSelect probe was originally derived was identified. Polymorphisms for *wsnp_Ra_rep_c117300_96881829* could only be detected using an SSCP gel (Figure 5A), whereas *wsnp_Ex_c16144_24583060* was converted to a KASP marker (Figure 5B). Primer sequences for both markers are shown in Appendix 4. The candidate markers were validated by re-screening the *Lr34* non-carriers, and the results were compared to the iSelect genotypic data. Both primers had perfect association with the iSelect assay data, providing validation for the markers, and confirming that both the new markers and the iSelect probes from which they were derived co-segregate (data not shown). Attempts were also made to develop PCR-based markers for the important stripe rust QTL *QYr.usw-6B* and *QYr.usw-4A*, but were not successful.

Table 6: Summary of results from multiple QTL mapping (MQM) for 2011 and 2012 field data. Traits measured included leaf rust DS Saskatoon (LRS) and Portage (LRP), stripe rust DS Lethbridge (YRL), coefficient of infection Saskatoon (CIS) and Portage (CIP), area under the disease progress curve Saskatoon (AUDPC), and leaf tip necrosis Saskatoon (LTN).

2011						
Trait	**Linkage Group**	**Peak Locus /Interval**	**Peak Position (cM)**	**R^2 (%)**	**LOD**	**Positive Allele**
LRS	*QLrs.usw-2B1*	*wsnp_Ex_c16144_24583060 - wsnp_Ra_rep_c117300_96881829*	45.7	25.3	11.25	A
	QLrs.usw-2B2	*Xwmc764*	0.0	7	3.76	A
LRP	*QLrp.usw-2B1*	*wsnp_Ex_c16144_24583060 - wsnp_Ra_rep_c117300_96881829*	45.7	41.7	13.54	A
YRL	*QYr.usw-6B*	*wsnp_Ex_c12618_20079758*	60.2	18.2	7.60	B
	QYr.usw-4A	*wsnp_Ex_c539_1072859*	58	9.2	3.51	A
	QYr.usw-2B1	*Wsnp_Ex_rep_c66551_64836462*	32.3	8.6	3.29	A
CIS	*QCis.usw-2B1*	*wsnp_Ex_c16144_24583060 - wsnp_Ra_rep_c117300_96881829*	49.7	30.8	11.50	A
	QCis.usw-4A	*wsnp_Ex_c4068_7351806*	51.3	8.6	3.92	A
CIP	*QCip.usw-2B1*	*wsnp_Ex_c16144_24583060 - wsnp_Ra_rep_c117300_96881829*	49.7	23.9	6.10	A
AUDPC	*QAudpc.usw-2B1*	*wsnp_Ex_c16144_24583060 - wsnp_Ra_rep_c117300_96881829*	49.7	43.3	13.30	A
	QAudpc.usw-2B2	*Xwmc764*	0.0	12.1	4.68	A
	QAudpc.usw-4A	*wsnp_Ku_c3081_5777013*	45.7	10.1	3.84	A
LTN	*QLtn.usw-2B1*	*wsnp_Ex_c16144_24583060 -wsnp_Ra_rep_c117300_96881829*	49.7	14.6	4.47	A

Positive Allele Source: A = AC Domain, B = RL4452

Table 6: Continued

	2012					
	Linkage Group	**Peak Locus/ Interval**	**Peak Position (cM)**	**R^2 (%)**	**LOD**	**Positive Allele**
LRS	*QLrs.usw-2B1*	*wsnp_Ex_c16144_24583060 - wsnp_Ra_rep_c117300_96881829*	45.7	38.9	13.64	A
LRP	*QLrp.usw-2B1*	*wsnp_Ex_c16144_24583060 - wsnp_Ra_rep_c117300_96881829*	45.7	21.8	6.67	A
	QLrp.usw-4A	*wsnp_Ex_rep_c67054_65517671*	33.7	12	3.93	A
YRL	*QYr.usw-6B*	*wsnp_Ex_c12618_20079758*	60.2	13.9	4.63	B
	QYr.usw-4A	*wsnp_Ra_c1022_2067517*	57.0	11	3.76	A
	QYr.usw-2B1	*wsnp_Ex_c16144_24583060 - wsnp_Ra_rep_c117300_96881829*	45.7	10.7	3.65	A
CIS	*QCis.usw-2B1*	*wsnp_Ex_c16144_24583060 - wsnp_Ra_rep_c117300_96881829*	49.7	36.3	12.24	A
	QCis.usw-3B	*wsnp_Ex_c18915_27811736*	62.6	7.1	3.05	A
	QCis.usw-1B	*Xwmc44*	37.2	7.1	3.03	B
CIP	*QCip.usw-2B1*	*wsnp_Ex_c16144_24583060 - wsnp_Ra_rep_c117300_96881829*	45.7	25	8.31	A
	QCip.usw-5B	*wsnp_Ku_c61976_63270478*	56.3	10.2	3.81	A
AUDPC	*QAudpc.usw-2B1*	*wsnp_Ex_c16144_24583060 - wsnp_Ra_rep_c117300_96881829*	45.7	42	14.51	A
	QAudpc.usw-3B	*Wsnp_Ex_c3130_5789888*	62.6	8.6	3.91	A
	QAudpc.usw-1B	*Xwmc44*	37.2	7.3	3.36	B
LTN	*QLtn.usw-2B1*	*wsnp_Ex_c16144_24583060 - wsnp_Ra_rep_c117300_96881829*	45.7	19.5	6.31	A

Positive Allele Source: A = AC Domain, B = RL4452

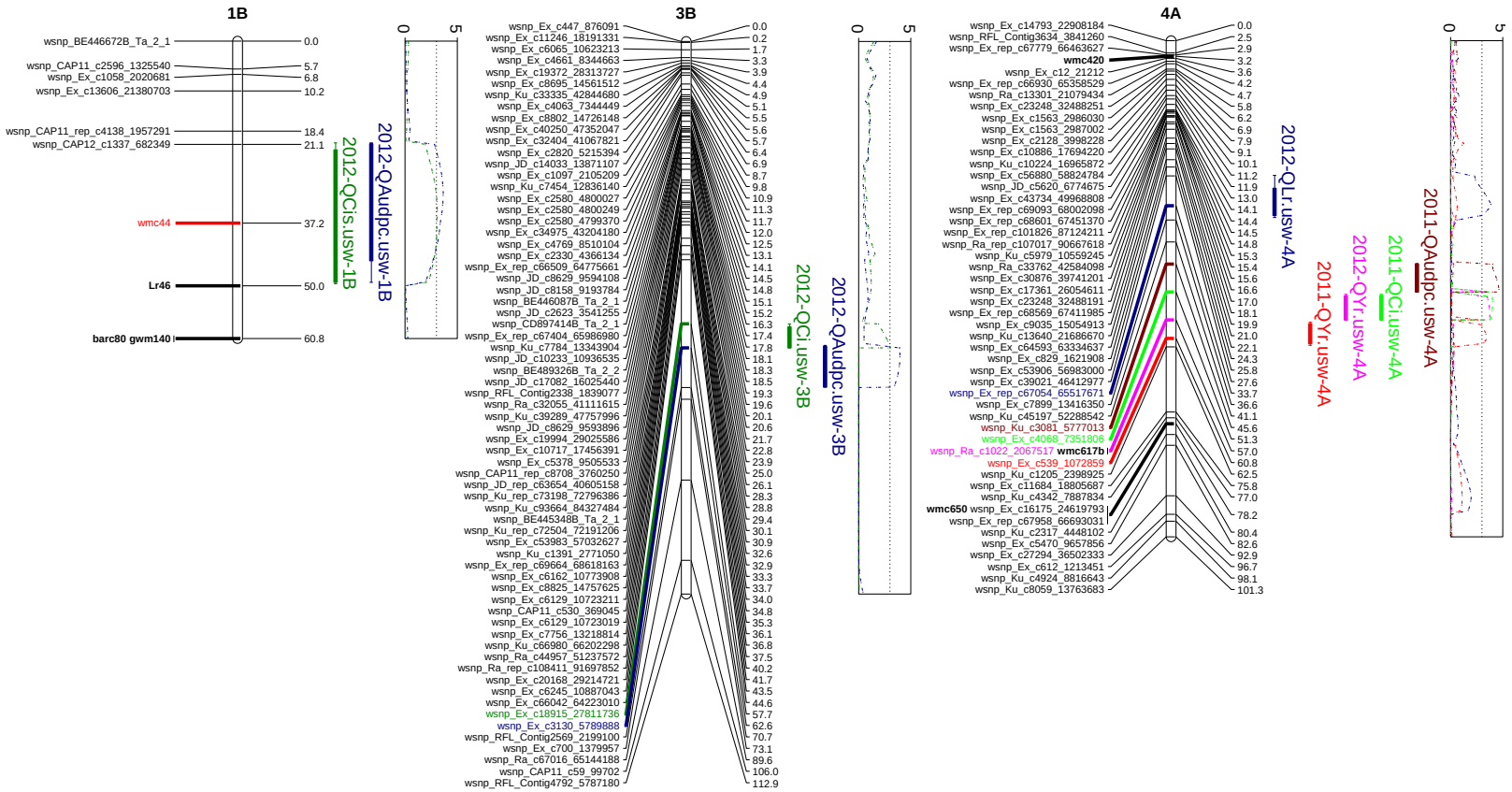

Figure 4: Linkage maps from the cross RL4452/AC Domain showing 28 leaf and stripe rust QTL across six chromosomes. Marker names are shown to the left of the linkage maps, and their corresponding map positions (cM) are shown in line to the right. Peak loci for each QTL are highlighted in color coded font. QTL graphs are shown to the right of linkage maps with LOD scores for each marker plotted along the Y-axis. The dashed line running across each graph represents the minimum threshold for declaring significant QTL.

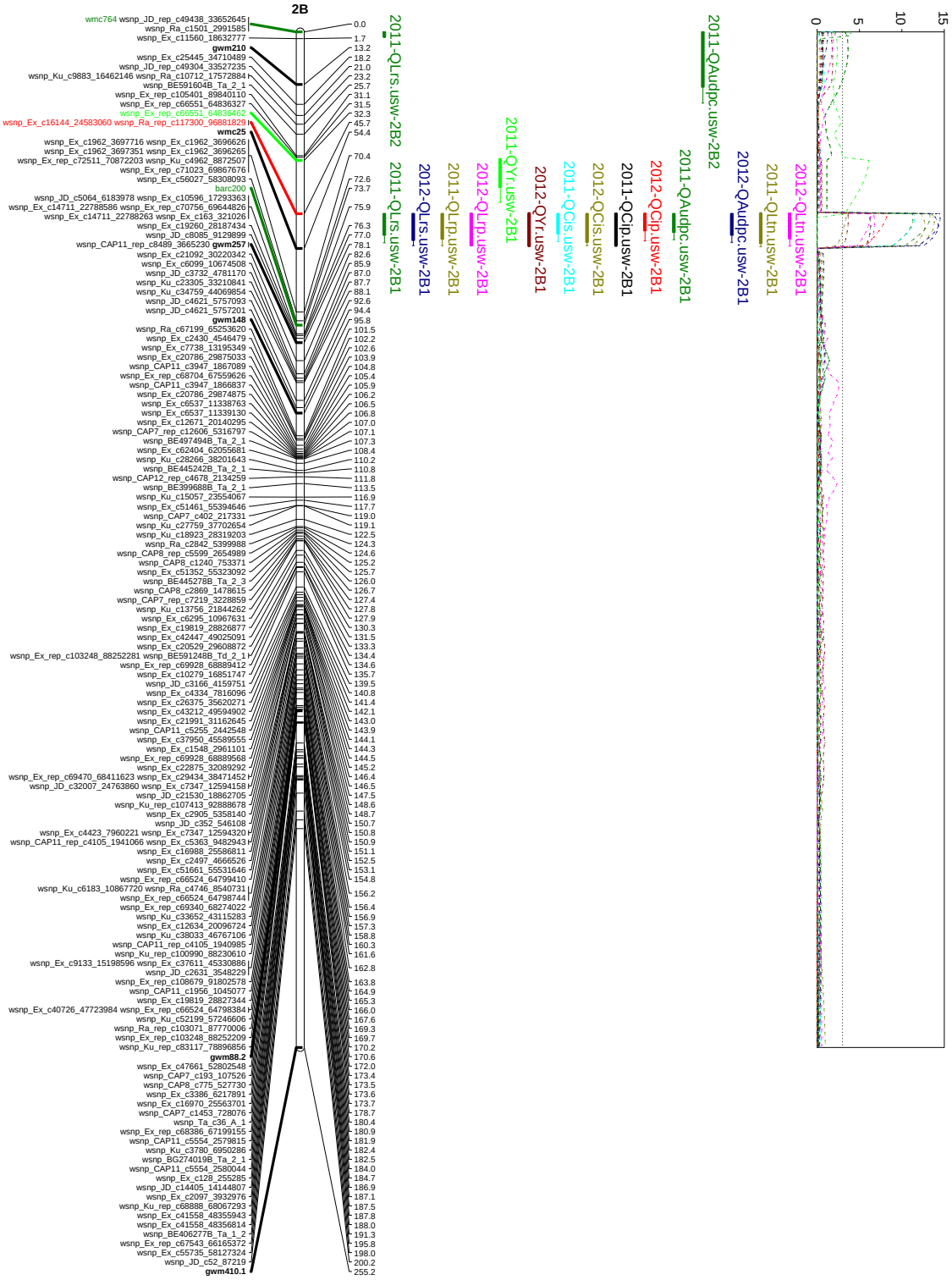

Figure 4: Continued

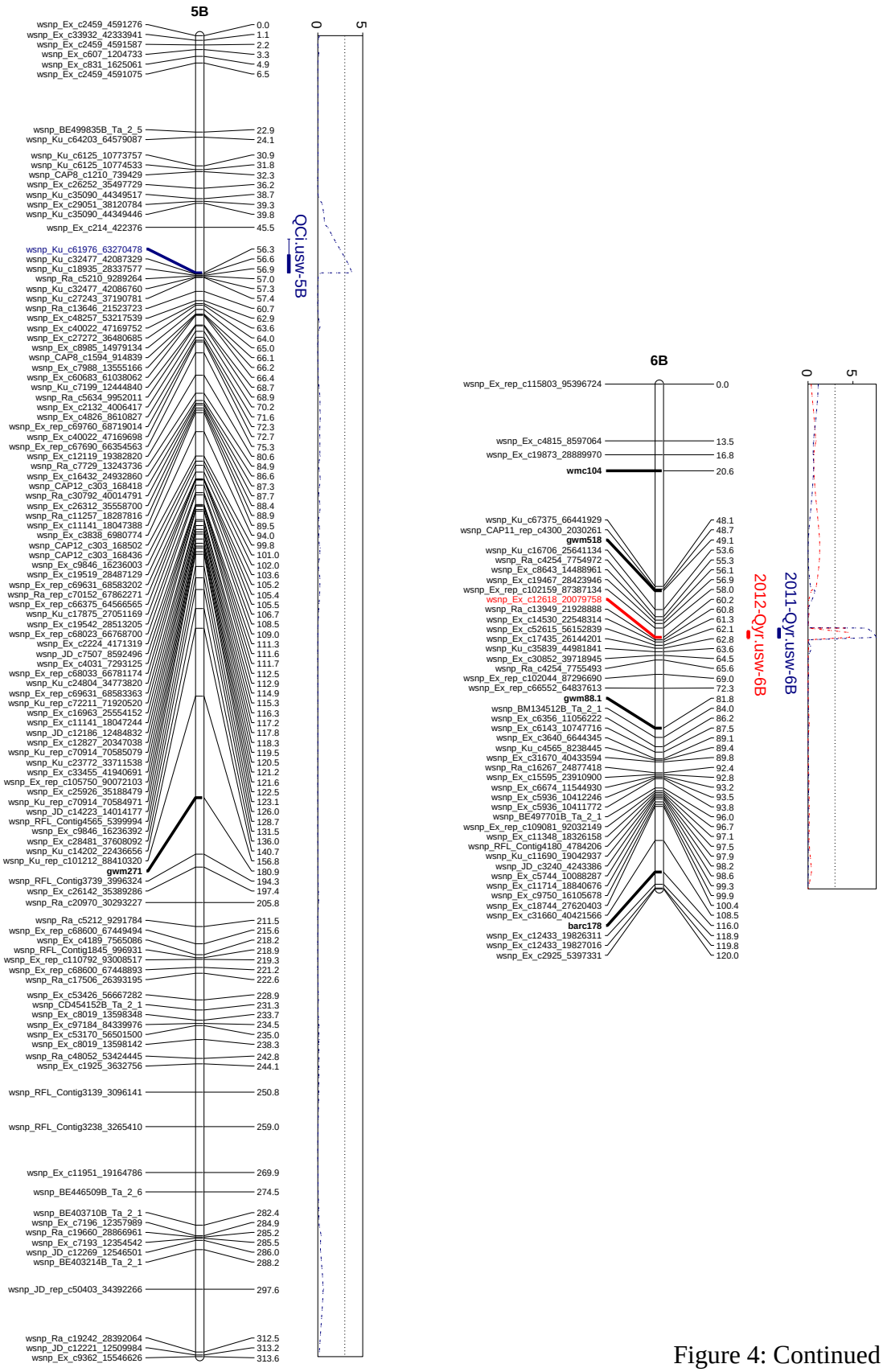

Figure 4: Continued

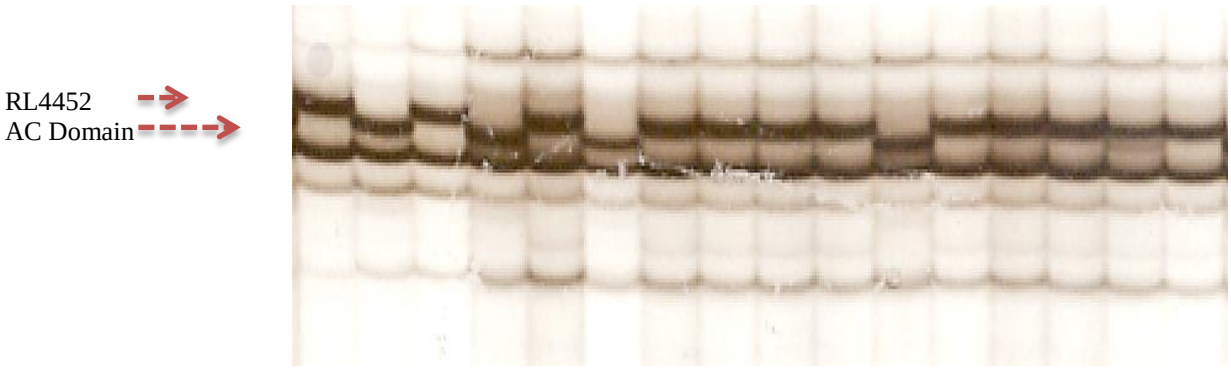

Figure 5A: Stained SSCP Gel showing the banding pattern of SSCP-SNP-wsnp_Ra_rep_c117300_96881829.

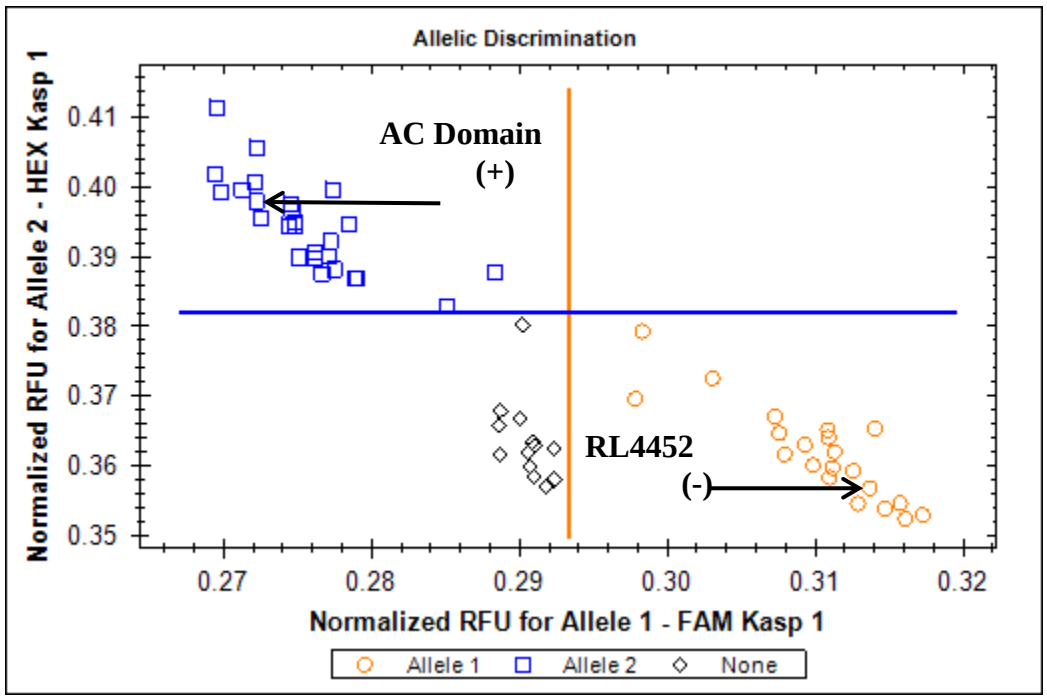

Figure 5B: Scoring of the *SNP-wsnp_Ex_c16144_24583060* KASP marker on a subset of lines in the DH mapping population. Check cultivars are shown with an arrow. Lines carrying the + marker allele separated into the upper left quadrant and included AC Domain, while lines carrying the - marker allele separated into the bottom right quadrant which included RL4452. The black triangles located in the bottom left quadrant were negative control samples containing no sample DNA.

SSCP-SNP-wsnp_Ra_rep_c117300_96881829 amplified one locus (thus two bands on an SSCP), with one band showing a clear polymorphism that distinguished RL4452 from AC Domain after silver staining the SSCP gel (Figure 5A). Additional faint bands were also visible on the gel, which were probably the result of background amplification of additional

monomorphic homeologous copies of the marker. *SSCPSNP-wsnp_Ra_rep_c117300_96881829* was put through further testing by screening a set of diverse hexaploid and tetraploid breeding lines (diversity panel). The results of the assay on that material are presented in Appendix 5.

Red Fife, one of the early CWRS wheat cultivars grown in North America and progenitor of many of today's modern wheat varieties, amplified the positive marker allele. Marquis, a line derived from Red Fife, amplified a unique band, which was likely due to the occurrence of a non-target SNP causing a conformational change within the amplicon. Interestingly, many of the lines derived from AC Domain, such as Kane, Superb and Stettler did not amplify the positive marker allele (Figure 6). Also derived from an AC Domain background, Waskada and Muchmore did amplify the positive marker allele. However, because pedigree analysis suggests AC Domain did not pass on the positive marker allele to its direct offspring, it is likely that Waskada and Muchmore inherited the locus from a source other than AC Domain (Figure 6). Roblin amplified the positive allele, along with most lines derived from a Roblin background including AC Splendor, AC Intrepid and Goodeve (Figure 7). Neepawa was not run in our analysis; however, many Neepawa derived lines carry the positive marker allele including CDC Teal, Katepwa, CDC Osler and Prodigy, which suggests that Neepawa might itself be a carrier (Figure 7). Glenlea did not amplify the positive marker allele, and likewise many lines derived from a Glenlea background such as RL4452, CDN Bison, CDC Walrus and CDC Rama also amplified the susceptible allele (Figure 8).

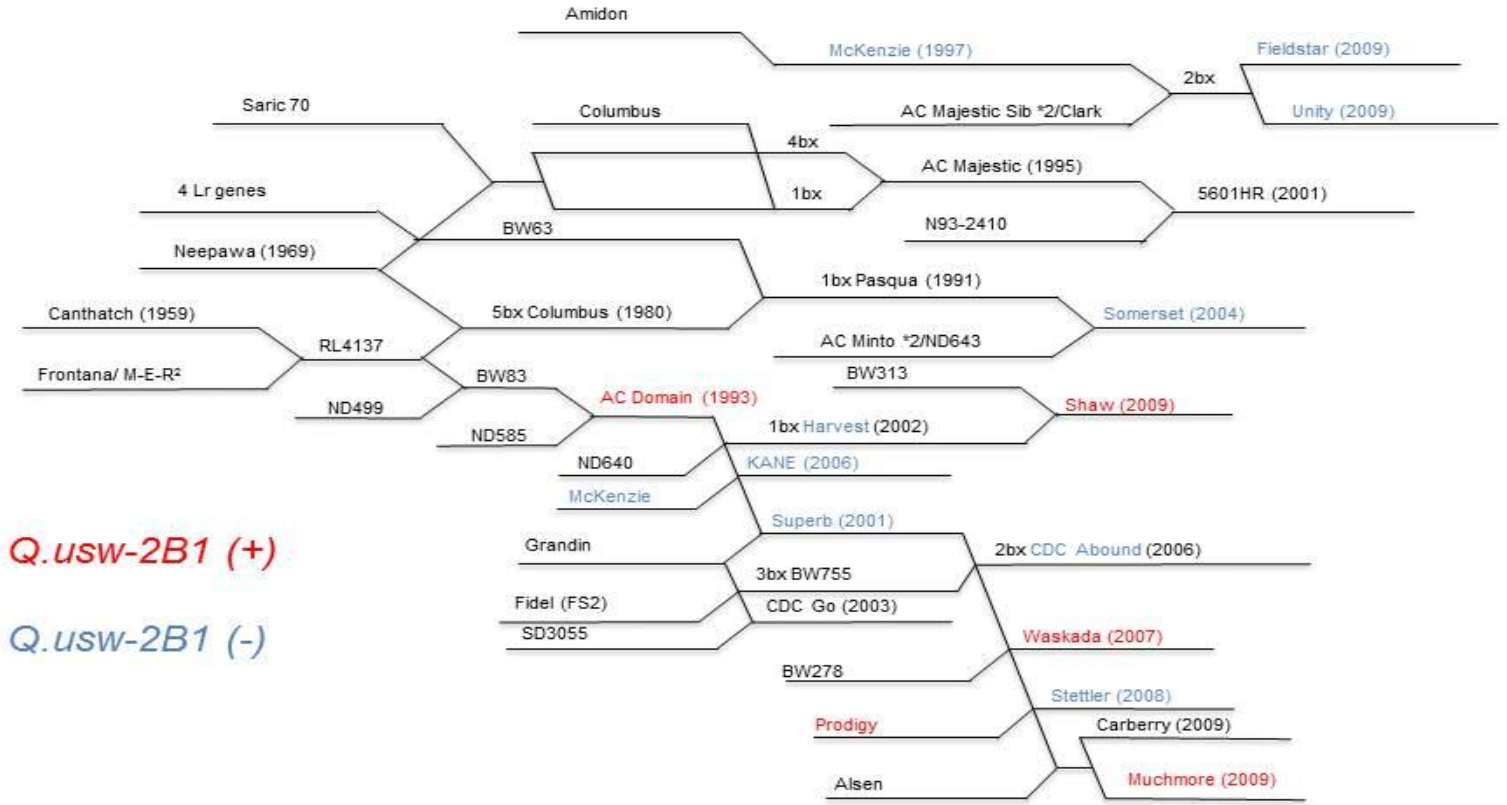

Figure 6: Pedigree relationships showing lines derived from an AC Domain background (adapted from McCallum et al. 2012a). Names highlighted in blue font carry the negative allele for *Q.usw-2B1*, while lines highlighted in red font carry the positive allele for *Q.usw-2B1*. Lines in black font were not run in the analysis.

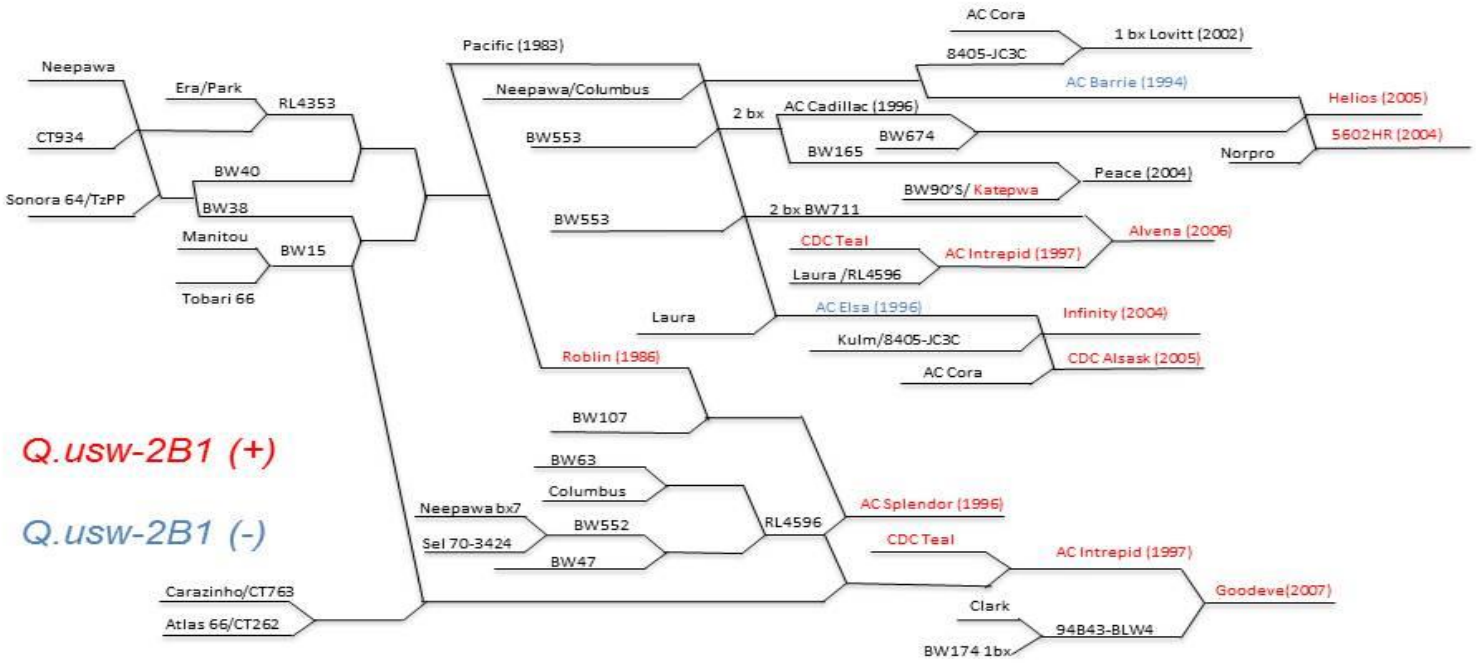

Figure 7: Pedigree relationship showing lines derived from a Neepawa/Roblin background (adapted from McCallum et al. 2012a). Names highlighted in blue font carry the negative allele for *Q.usw-2B1*, while lines highlighted in red font carry the positive allele for *Q.usw-2B1*. Lines in black font were not run in the analysis.

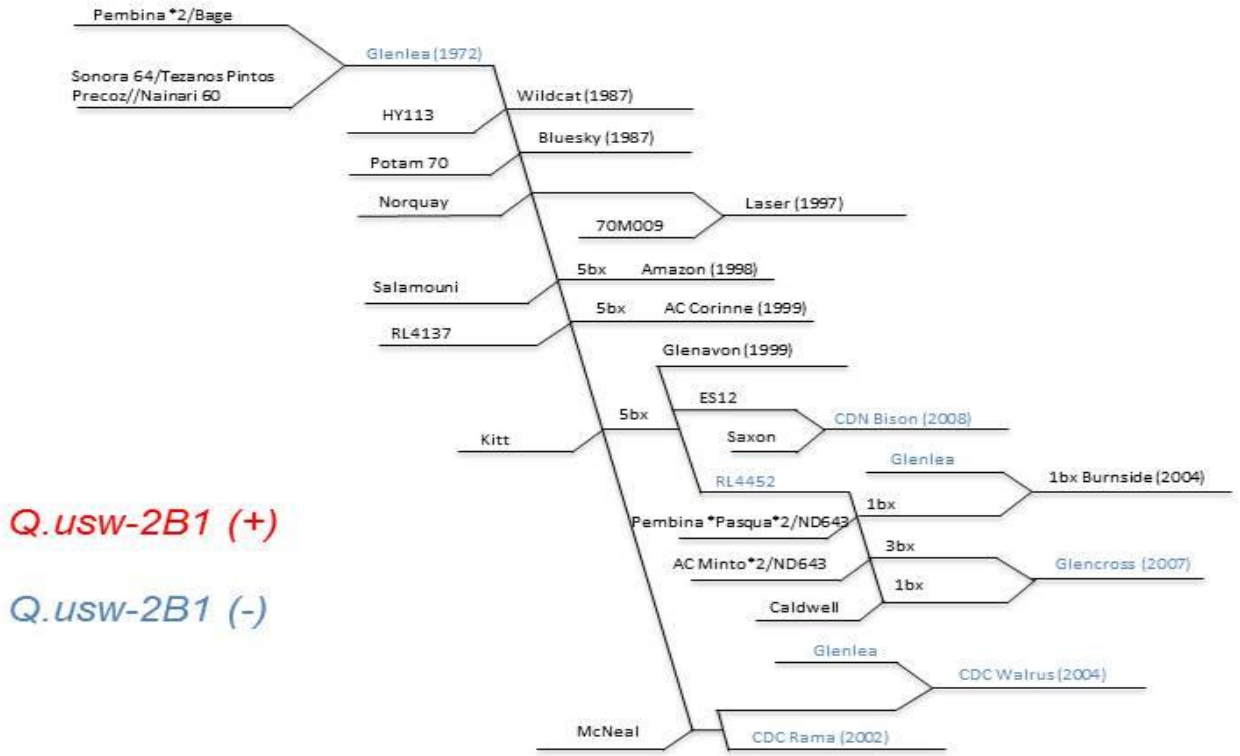

Figure 8: Pedigree relationship showing lines derived from a Glenlea Background (adapted from McCallum et al. 2012a). Names highlighted in blue font carry the negative allele for *Q.usw-2B1*, while lines highlighted in red font carry the positive allele for Q.usw-2B1. Lines in black font were not run in the analysis.

SNP-wsnp_Ex_c16144_24583060 proved to be an excellent KASP marker that amplified two easily distinguishable groups in the RL4452 /AC Domain population (Figure 5B). However, when the marker was tested on the diversity panel, the results were difficult to interpret (data not shown), therefore the marker would not be robust for applications involving screening of diverse lines. Of the two, *SSCPSNP-wsnp_Ra_rep_c117300_96881829* is probably the best candidate for marker-assisted selection since it proved to be more robust on a diverse set of lines. That being said, *SNP-wsnp_Ex_c16144_24583060* is more user-friendly, and may still be useful for applications involving mapping populations where comparison between parental genotypes can be achieved.

4.5 Assessing the Combined Effects of Rust Resistance Loci in the RL4452 x AC Domain Population

Because the iSelect 9K assay was only run on the *Lr34* non-carriers, the PCR-based markers: *SNP-wsnp_Ex_c16144_24583060* and *SSCP-SNP-wsnp_Ra_rep_c117300_96881829* were run on the entire mapping population to test for interactions among *Q.usw-2B1*, *Lr34*, *Lr16*, and *Lr46*. For all subsequent statistical analyses, the genotypic data obtained from the marker *SSCP-SNP-wsnp_Ra_rep_c117300_96881829* was used as a representative SNP to estimate the phenotypic effects of *Q.usw-2B1*.

4.5.1 Saskatoon

An ANOVA for the combined analysis of the 2011-2012 Saskatoon phenotypic data with combined genotypic data was conducted and is presented in Appendix 6. For this analysis, *Xwmc764* was diagnostic for *Lr16*. Over both field seasons, the main effects of *Lr34* and *Q.usw-2B1* were significant (P<0.001) for DS, CIS, and AUDPC. Similarly, a significant main effect (P<0.001-0.01) of *Xwmc764 (Lr16)* was detected for all three traits. Across both years of testing, a significant interaction between *Lr34* and *Q.usw-2B1* was detected for DS, CIS and AUDPC (P<0.001 – 0.05). The interaction between *Lr34* and *Lr16* was also significant (P<0.05) for these three traits in 2011, but was not significant in 2012. In 2011, the interaction between all four loci was significant for DS (P<0.05).

The four way interaction for 2011 Saskatoon DS was complex (Figure 9). As expected, experimental lines that carried no resistance alleles scored that highest DS of the allelic combinations. Clearly, *Lr34* had the strongest effect of the four loci, since the mean DS

expressed by carriers of the gene did not exceed 10%. In general, the presence of *Lr34* reduced DS to its lowest significant level masking the potential expression of the other loci. One important exception to this was that in the *Lr34* non-carriers, lines that did carry all three positive alleles for *Lr16*, *Lr46* and *Q.usw-2B1* expressed a statistically equivalent level of resistance to the resistance provided by *Lr34* (Figure 9). Although in many cases significant differences were not detectable, there was an overall trend of decreasing DS with the accumulation of resistance genes. In 2012, where disease pressure at Saskatoon was higher than the previous year (mean population DS = 20.1%; Table 4), the differentiation between genotypic classes was greater (Table 4). Similar to 2011 data, lines lacking both *Lr34* and *Q.usw-2B1* scored the highest DS (DS=40%, Figure 10). The presence of one positive allele for either of *Lr34* or *Q.usw-2B1* had an equivalent effect in reducing DS by approximately 50 % compared to lines with no resistance alleles for either locus. Most importantly, the presence of both resistance alleles decreased disease severity by up to 75% compared to lines with no resistance alleles. The same pattern was also apparent for CIS 2012 (Figure 11).

Analysis of the 2011 CIS and AUDPC data revealed that only in the absence of *Lr34* was the effect of *Q.usw-2B1* significant in reducing both trait scores (Figures 11,12). In lines carrying *Lr34*, trait values were reduced to their lowest significance level and the effect of *Q.usw-2B1* did not further reduce CIS or AUDPC scores (Figures 11,12). Results showed AUDPC 2012 had statistically significant disease effects for each of the four possible allelic combinations (Figure 12). When neither resistance allele was present, AUDPC was highest (AUDPC=164). The effect of *Lr34* in the absence of *Q.usw-2B1* was stronger than the effect of *Q.usw-2B1* in the absence of *Lr34*. When both resistance alleles were present AUDPC was the lowest (AUDPC= 42.7).

4.5.2 Portage

An ANOVA table summarizing the results from the analysis of the 2011-2012 Portage field data with combined genotypic data is presented in Appendix 7. At Portage, *Lr34* was significantly associated ($P<0.001$) with DS and CIP in 2011 and 2012, respectively. Similarly the main effect of *Q.usw-2B1* was significant ($P<0.001$-0.01) for both traits over two years. A significant interaction was detected between *Lr34* and *Q.usw-2B1* for both years and traits, except for 2012 DS where the interaction was not significant. The Portage 2011 results showed that only in the absence of *Lr34* was the effect of *Q.usw-2B1* significant in reducing DS and CIP (Figures 13,15), and the same pattern was observed for CIP 2012 (Figure 15). Portage 2012 DS

was influenced by the main effects of *Lr34* and *Q.usw-2B1*. Results showed that *Lr34* had a large effect in reducing DS by up to 40% between non-carriers and carriers. In contrast, *Q.usw-2B1* did not have a large effect, only reducing disease around 8% between carriers and non-carriers (Figure 14).

4.5.3 Lethbridge

An ANOVA table summarizing the results from analysis of the 2011-2012 Lethbridge field data with combined genotypic data is presented in Appendix 8. Analysis of the overall dataset revealed that *Lr34* was the single significant ($P<0.001$) factor controlling stripe rust DS at Lethbridge in 2011-2012. In both years, the *Lr34* non-carriers averaged close to 40 % DS. The *Lr34* carriers performed well, and averaged 10 and 21 % DS in 2011 and 2012, respectively (Figure 16).

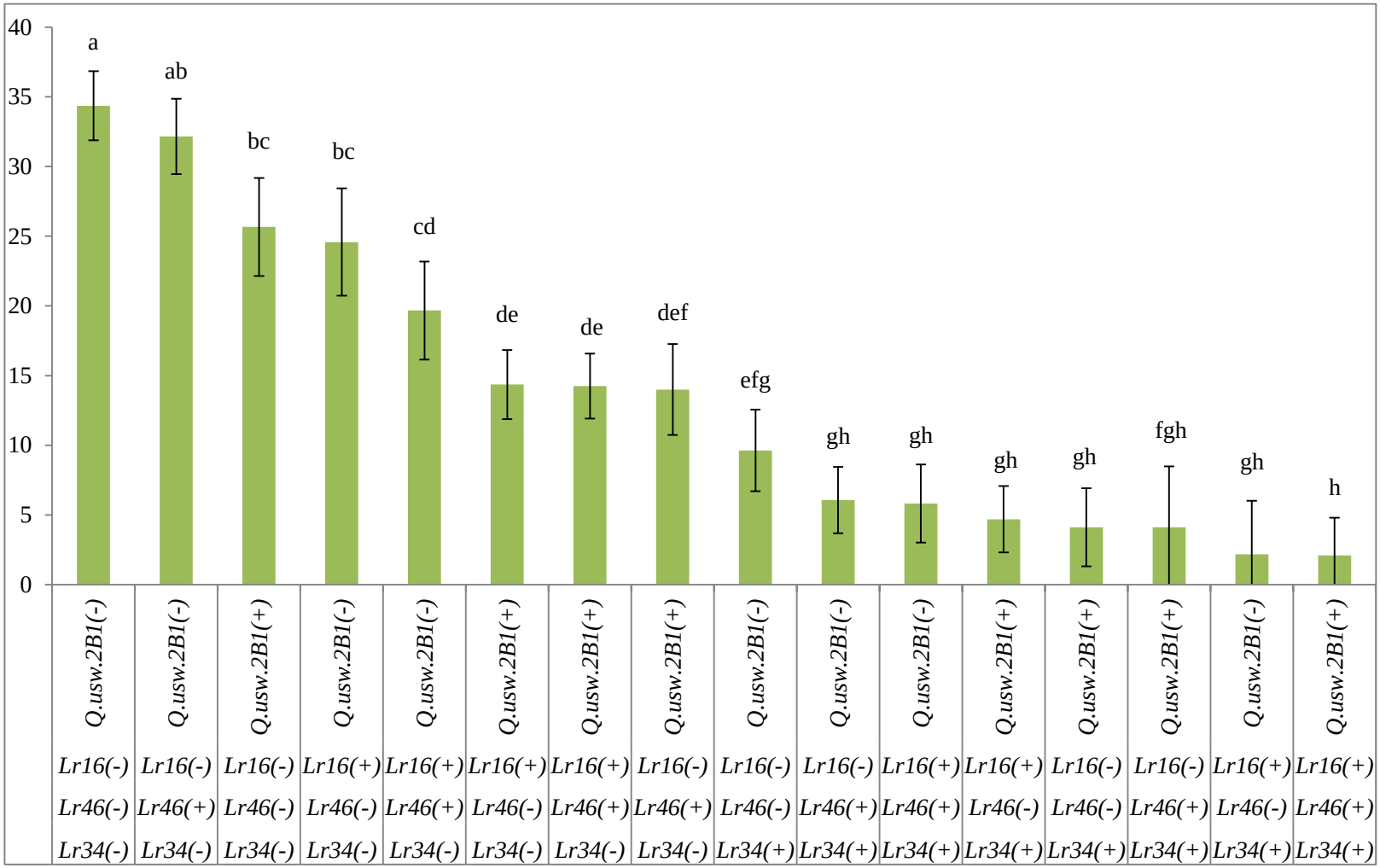

Figure 9: LS means showing the four way interaction between *Q.usw-2B1*Lr34*Lr46*Lr1*6 for 2011 Saskatoon disease severity (DS). The X-axis shows each of the 16 possible four-way interactions between loci expected in the mapping population. Mean separation was performed using Fishers LSD test. Standard errors for each group are indicated by the error bars. Letters above bars indicate statistical significance at $P<0.01$.

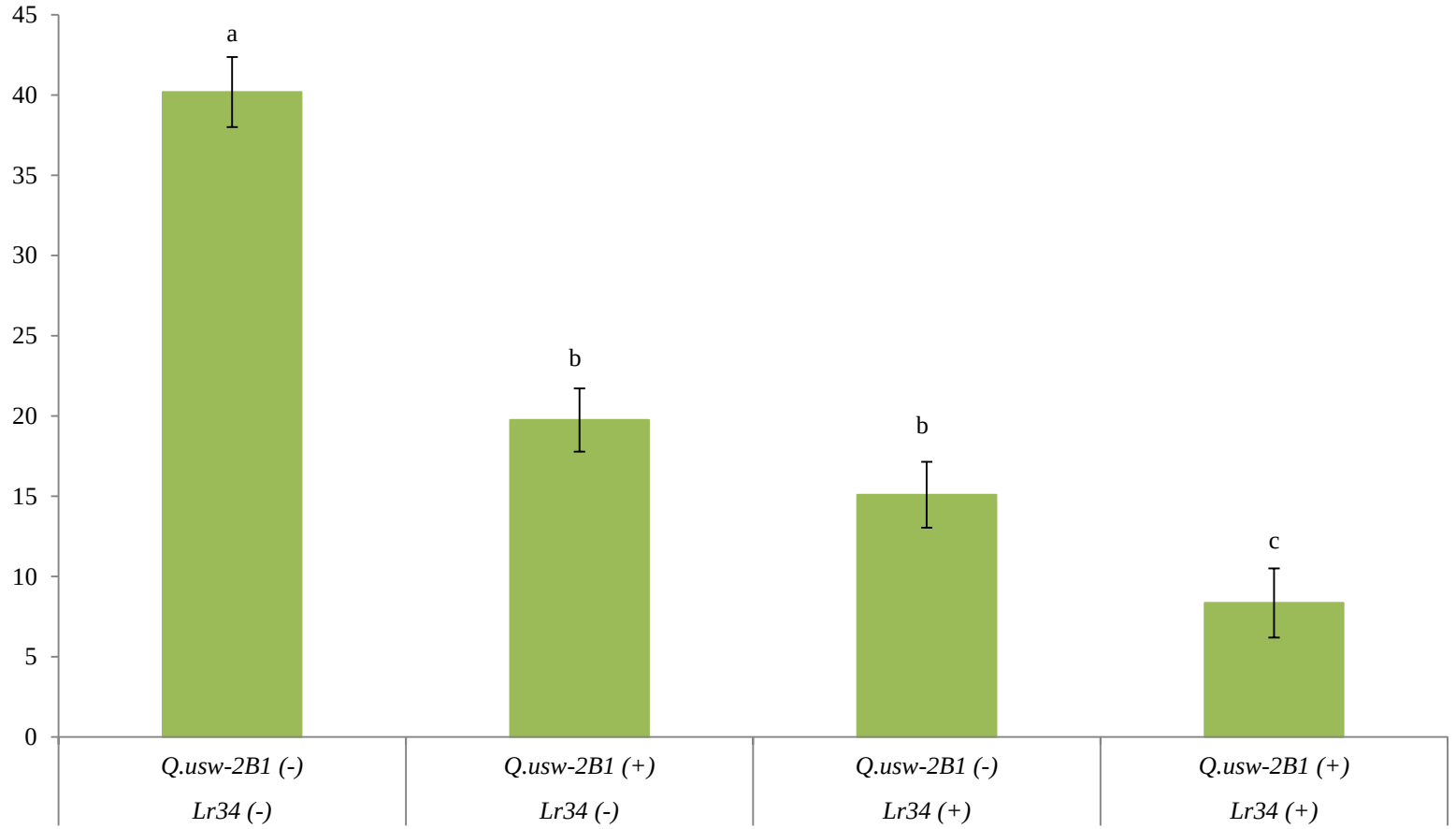

Figure 10: LS means showing the two-way interaction between *Q.usw-2B1*Lr34* for 2012 Saskatoon disease severity (DS). The X-axis of the graph shows each of the four possible allelic combinations expected in the mapping population. Mean separation was performed using Fishers LSD test. Standard errors for each group are indicated by the error bars. Letters above bars indicate statistical significance at $P<0.01$.

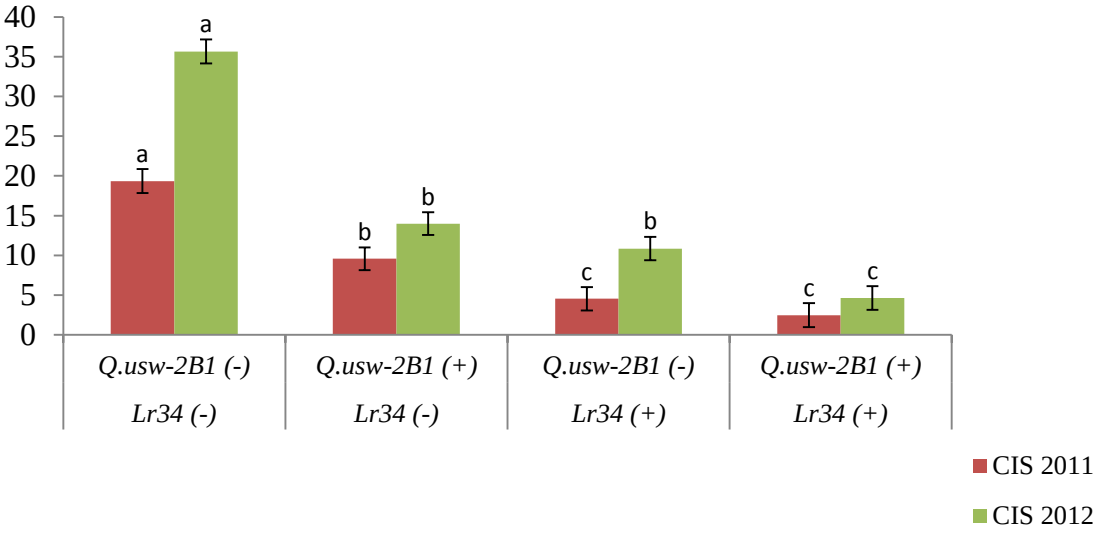

Figure 11: LS means showing the two-way interaction between *Q.usw-2B1*Lr34* for 2011-2012 Saskatoon coefficient of infection (CIS). The X-axis of the graph shows each of the four possible allelic combinations. Mean separation was performed using Fishers LSD test. Standard errors for each group are indicated by the error bars. Letters above bars indicate statistical significance at $P<0.01$. 12

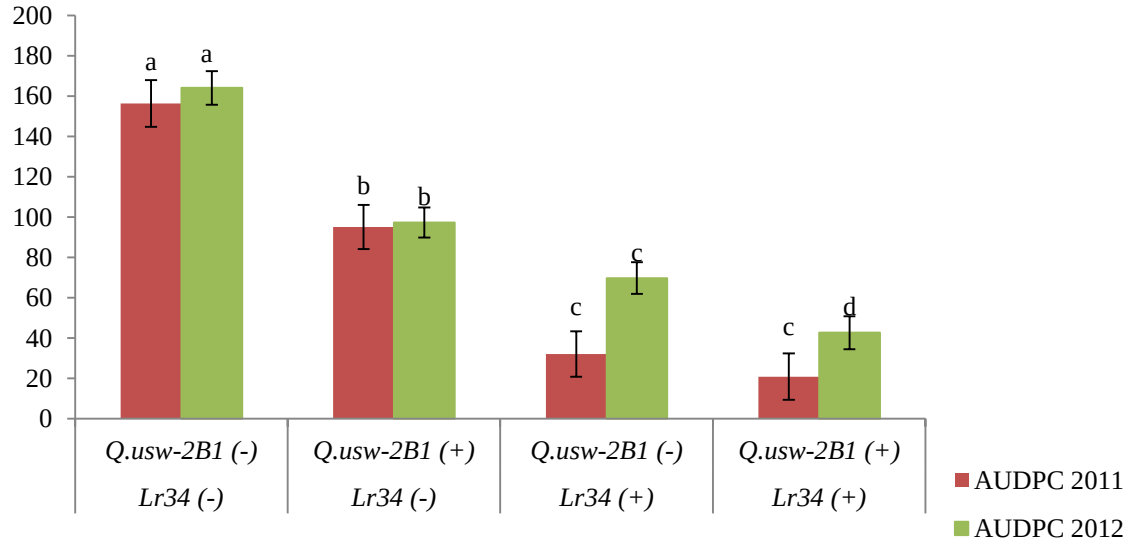

Figure 12: LS means showing the two-way interaction between *Q.usw-2B1*Lr34* for 2011-2012 Saskatoon area under the disease progress curve (AUDPC). The X-axis shows each of the four possible allelic combinations. Mean separation was performed using Fishers LSD test. Standard errors for each group are indicated by the error bars. Letters above bars indicate statistical significance at $P<0.01$.

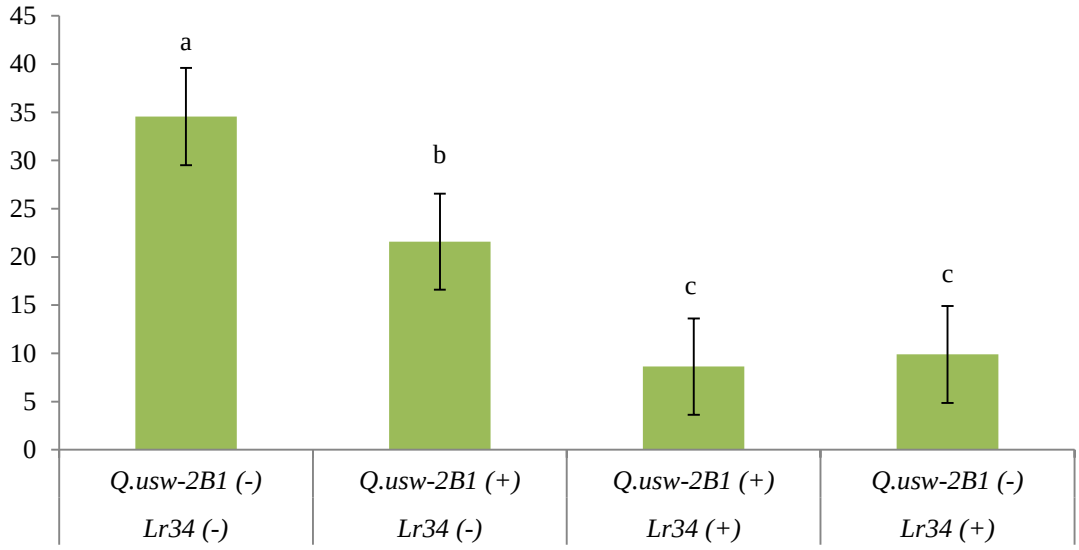

Figure 13: LS means showing the two-way interaction between *Q.usw-2B1*Lr34* for 2011 Portage disease severity (DS). The X-axis of the graph shows each of the four possible allelic combinations. Mean separation was performed using Fishers LSD test. Standard errors for each group are indicated by the error bars. Letters above bars indicate statistical significance at $P<0.01$.

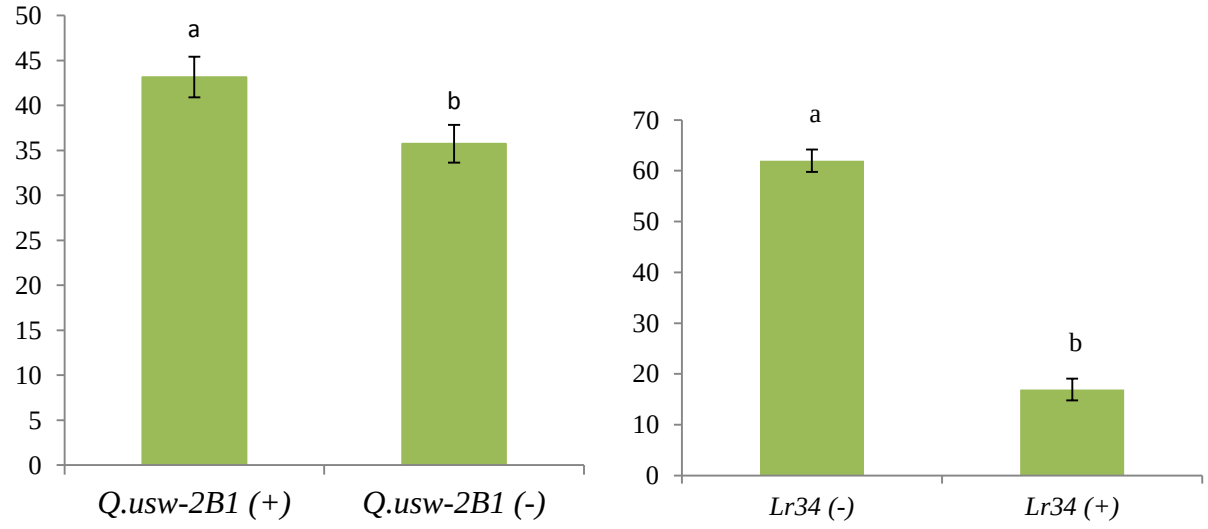

Figure 14: LS means showing the main effects of *Q.usw-2B1* and *Lr34* for 2012 Portage disease severity (DS). The X-axis of the graph shows each of the possible allelic combinations. Mean separation was performed using Fishers LSD test. Standard errors for each group are indicated by the error bars. Letters above bars indicate statistical significance at $P<0.01$.

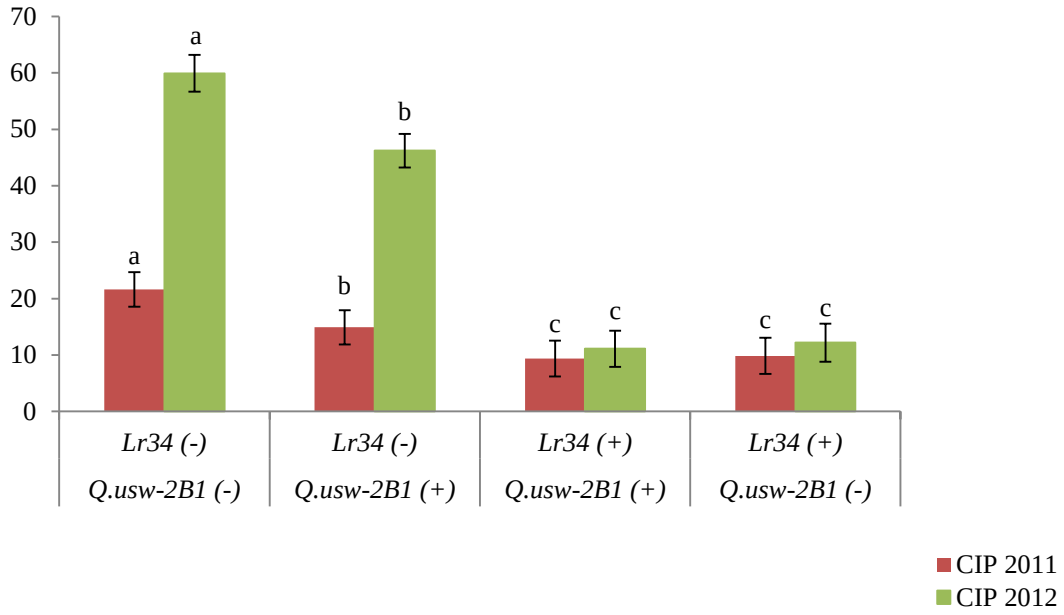

Figure 15: LS means showing the interaction effect between *Q.usw-2B1 * Lr34* for 2011-2012 Portage coefficient of infection (CIP). The X-axis of the graph shows each of the four possible allelic combinations. Standard errors for each group are indicated by the error bars. Letters above bars indicate statistical significance at $P<0.01$.

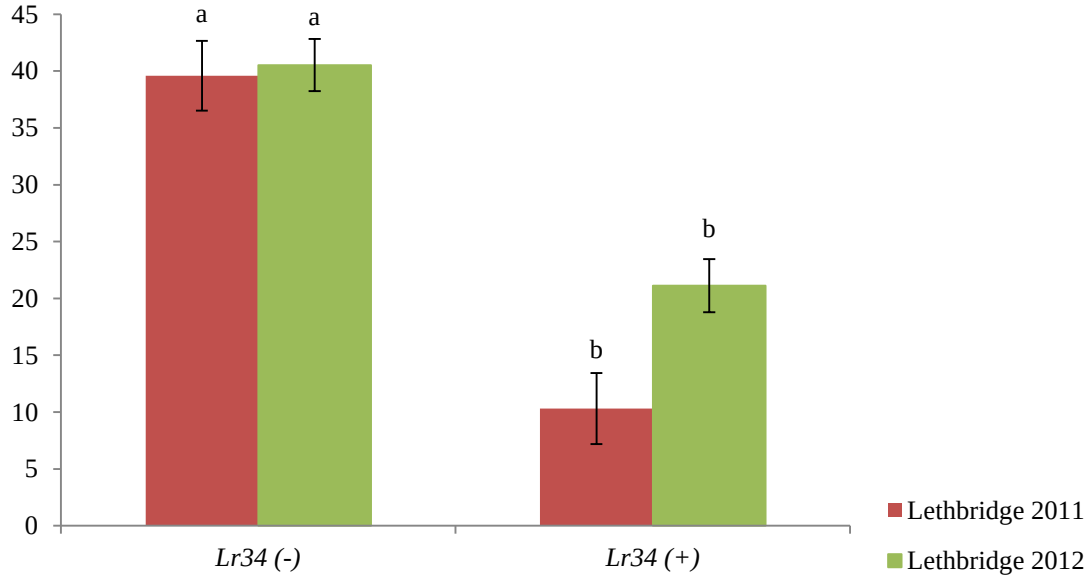

Figure 16: LS Means showing the main effect of *Lr34* for 2011-2012 Lethbridge disease severity (DS) The X-axis of the graph is each of the possible allelic combinations. Mean separation was performed using Fishers LSD test. Standard errors for each group are indicated by the error bars. Letters above bars indicate statistical significance at $P<0.01$.

4.5.4 Leaf Tip Necrosis

A key feature of *Lr34* and other reported APR genes in wheat is that their presence is strongly associated with expression of leaf tip necrosis (LTN). In an attempt to study possible effects of multiple APR genes on LTN severity, a rating scale of 0-5 was used, where 0= no visible signs of LTN, 5= Severe LTN. At Saskatoon, experimental lines expressed a range in severity for the LTN phenotype, and there were significant differences between lines (Appendix 6). Results from 2011-2012 showed that *Lr34* was significantly associated with the LTN phenotype ($P< 0.001$, Appendix 6), however, there were some lines that carry *Lr34* but did not express severe leaf tip necrosis (data not shown). In 2011, there was a significant 4-way interaction among *Lr34*, *Lr46*, *Lr16* and *Q.usw-2B1* influencing LTN severity (Appendix 6). Results showed that *Lr34* clearly had the strongest effect on LTN expression because when the gene was present LTN severities exceeded a score of 2.4 (Figure 17). Interestingly, the accumulation of positive alleles for all four loci appeared to increase LTN severity, although statistically significant differences between allelic classes were not always detectable (Figure 17). In 2012, only significant main effects were detected for *Lr34*, *Lr16* and *Q.usw-2B1* influencing LTN severity (Appendix 6). Results showed that lines carrying *Lr34* expressed the highest LTN severity (average LTN= 2.8), followed closely by lines carrying *Q.usw-2B1* (average LTN=2.7) and then by *Lr16* (average LTN= 2.6, Figure 18).

LTN was significantly associated ($P<0.001$) with DS, AUDPC and CI over the course of the study (Appendix 9). Interestingly, increasing LTN severity always resulted in a decrease in DS, AUDPC and CI (Table 7). These results were also reflected by the negative correlations between LTN and all trait scores that were detected across all testing environments (Table 5).

Table 7: Summary of results from Fisher's protected LSD test based on significant ANOVA F-tests for the main effect of LTN on Saskatoon leaf rust disease severity (DS), area under the disease progress curve (AUDPC) and coefficient of infection (CIS). All estimates are given with their associated standard errors.

	2011						2012					
LTN	**DS**	**S.E.**	**AUDPC**	**S.E.**	**CIS**	**S.E.**	**DS**	**S.E.**	**AUDPC**	**S.E.**	**CIS**	**S.E.**
0	22.796^{a}	2.927	131.21^{a}	16.133	16.580^{a}	2.175	41.544^{a}	1.845	171.97^{a}	7.205	37.324^{a}	1.819
1	20.641^{a}	2.734	117.10^{a}	14.857	14.166^{a}	2.022	18.898^{b}	1.537	84.384^{b}	6.043	13.812^{b}	1.515
2	15.378^{b}	2.502	82.948^{b}	13.294	9.865^{b}	1.837	19.533^{b}	1.734	86.782^{b}	6.786	14.348^{b}	0.688
3	12.114^{c}	2.468	69.370^{b}	13.064	7.712^{c}	1.810	17.023^{b}	1.340	81.525^{b}	5.303	11.840^{bc}	1.321
4	2.818^{d}	2.828	16.058^{c}	15.483	1.719^{d}	2.097	12.640^{c}	1.613	64.163^{c}	6.339	8.171^{cd}	1.59
5	0.285^{d}	4.591	0^{c}	26.730	0.277^{d}	3.476	8.464^{c}	2.033	47.660^{c}	7.924	4.371^{d}	2.00

Letters in superscript denote statistical significance ($p < 0.05$)

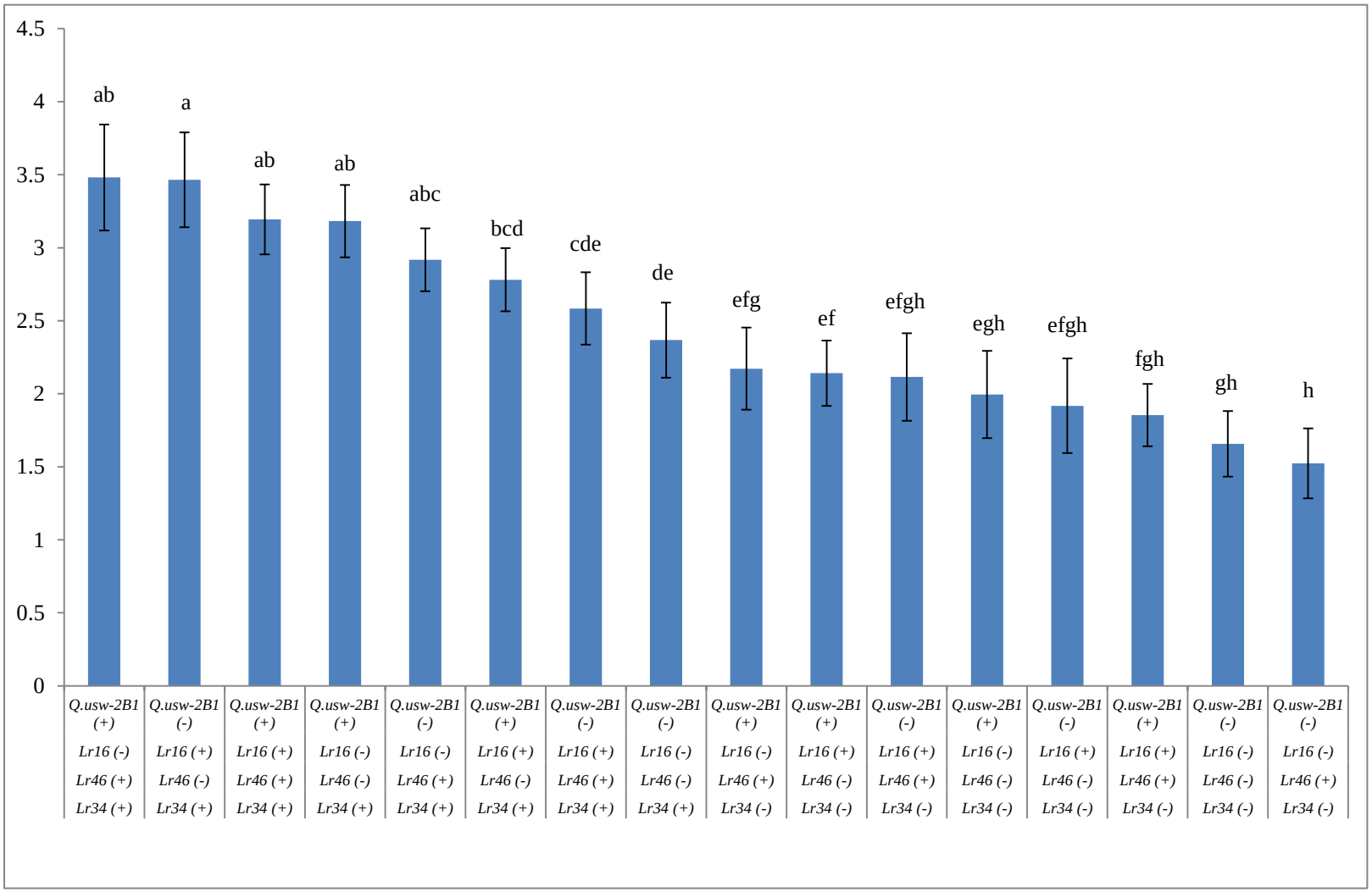

Figure 17: LS Means showing the four-way interaction between *Q.usw-2B1*Lr34*Lr46*Lr16* for 2011 LTN. The X-axis shows each of the 16 possible allelic combinations. The Y-axis shows LTN (0-5 Scale). Mean separation was performed using Fishers LSD test. Standard errors for each group are indicated by the error bars. Letters above bars statistically significant differences between groups at $P<0.05$.

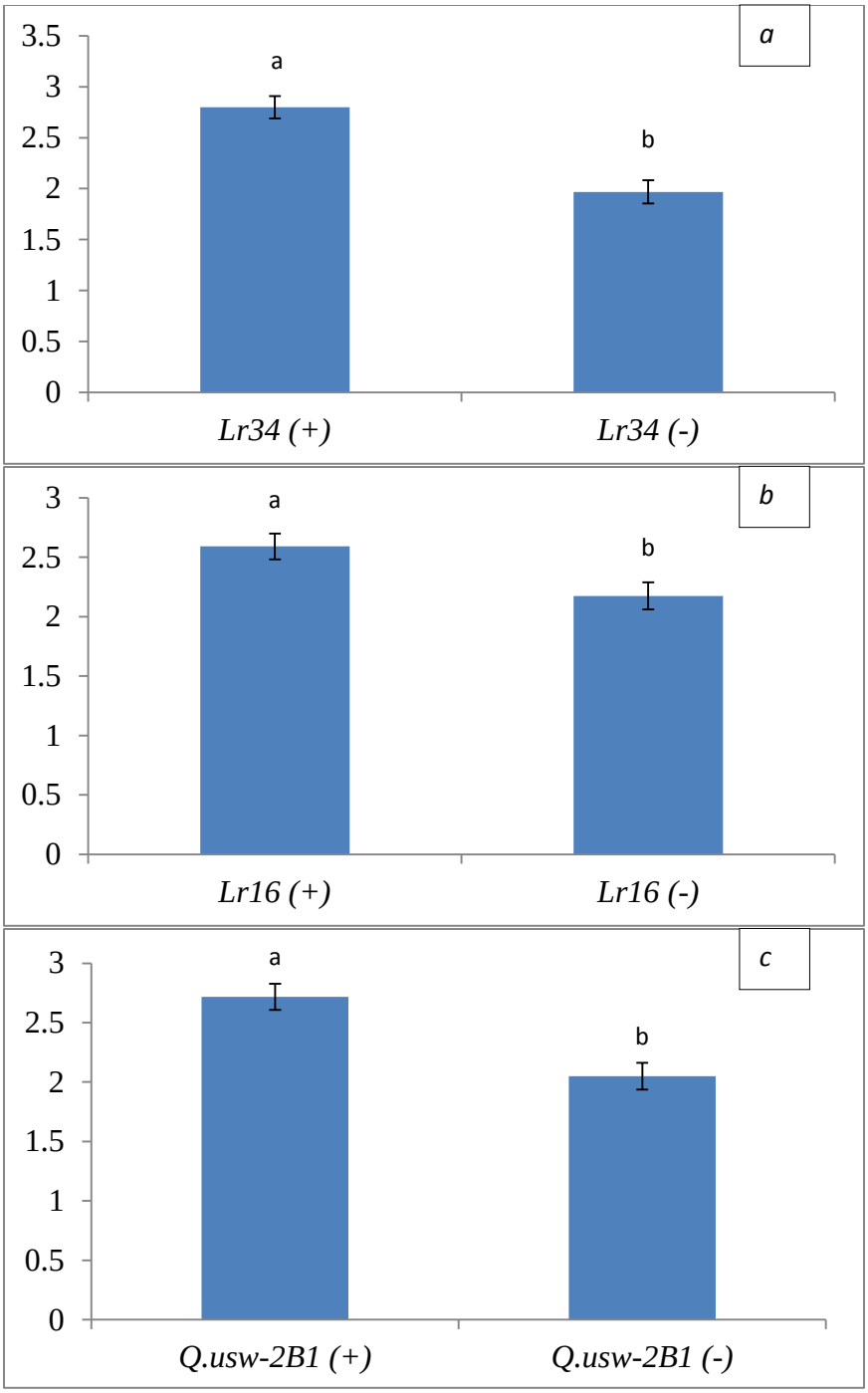

Figure 18: a-c: Mean leaf tip necrosis severity between a) *Lr34* carriers and non-carriers, b) between *Lr16* carriers and non-carriers and c) between *Q.usw-2B1* carriers and non-carriers. The X-axis shows different alleles for each locus. The Y-axis shows LTN severity (Scale = 0-5). Mean separation was performed using Fishers LSD test. Standard errors for each group are indicated by the error bars. Letters above bars indicate statistical significance at $P<0.05$.

4.6 Phytotron Seedling Rust Tests

4.6.1 Seedling Leaf Rust

In this study, the resistance at *Q.usw-2B1* conferred by AC Domain appeared to be behaving similar to *Lr34*, in that APR to leaf and stripe rust, and expression of LTN were associated with this QTL. In addition, the QTL was also associated with AUDPC at Saskatoon, which suggests a slowing of disease progression, another key defining feature of APR. Indoor seedling leaf rust evaluation was performed to test for the presence of additional all-stage, seedling resistance genes. Leaf rust infection types recorded during the experiment are presented in Table 8. The predominant infection type (IT) for Thatcher and RL4452 was IT= 4, indicating the complete susceptibility of these two lines at the seedling stage. These pustules were large and were not associated with any chlorosis or necrosis (Figures 19a, 19d, 20). Both AC Domain and Thatcher-*Lr16* expressed a mixed reaction IT that was phenotypically similar between the two lines (Figures 19b, 19c, 20). The predominant IT recorded on AC Domain was IT=3 but some pustules ranged up to a score of IT= 4 (Figures 19c, 20). Similarly, the predominant IT on Thatcher-*Lr16* was IT=3+ with some pustules scoring up to IT=4 (Figure 19b). Although some degree of host response was apparent on both lines, most pustules appeared quite healthy and were actively sporulating at the time of rating.

Table 8: Seedling leaf rust infection types recorded on four different lines in the indoor phytotron experiment.

ID	Score
AC Domain	3, 3+ , 4
RL4452	4, 3,3+
Thatcher-*Lr16*	3+,3,4
Thatcher	4,3+,3

Note: ratings are given in descending order beginning with the predominant infection type

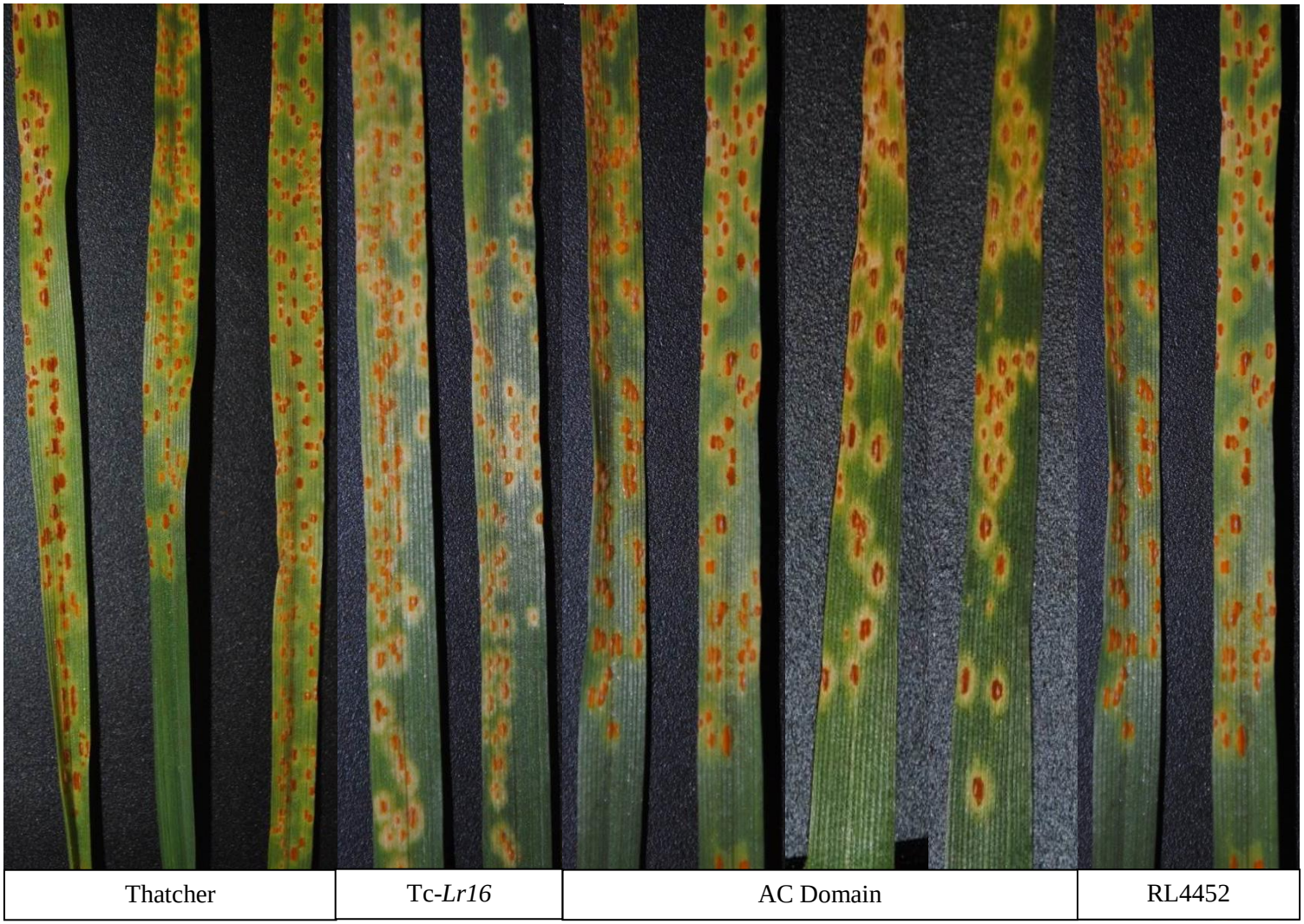

Figure 19 : Leaf rust infection type(s) recorded on**: Thatcher:** medium to large pustules with no chlorosis or necrosis (3, 4); **Thatcher-*Lr16*:** small sized pustules with chlorotic/necrotic rings (2), varying intermediate pustules with chlorosis (2-3), Medium + sized pustules with no chlorosis (3+)**; AC Domain:** large pustules with no chlorosis (4), varying intermediate sized pustules with or without chlorosis (3+), medium –large pustules with chlorosis (3) large pustules with minor chlorosis (3+); and **RL4452**: medium pustule size with or without light chlorosis (3+), large pustules with no chlorosis (4)

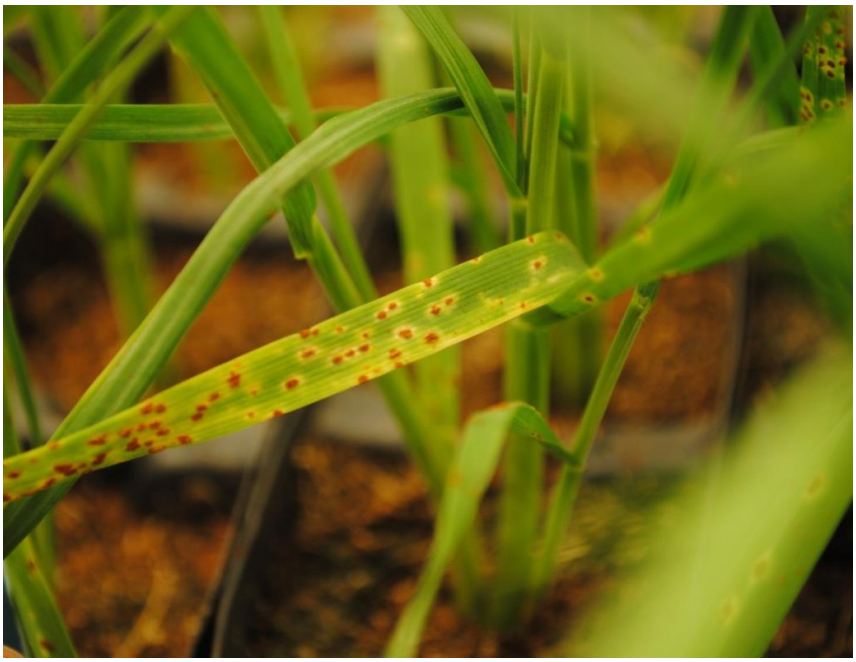

Figure 20: Leaf rust infection type(s) recorded on AC Domain: mixed infection type. Medium pustules surrounded by chlorosis (3), necrotic flecking (;) and large pustules with no chlorosis (4).

Figure 21: Leaf rust infection type(s) recorded on RL4452: large pustules no chlorosis or necrosis (4)**.**

4.6.2 Seedling Stripe Rust

Analysis of the field stripe rust data indicated the possibility of seedling gene(s) segregating in the population. However, seedling testing revealed that both AC Domain and RL4452 were susceptible to the mixture of Lethbridge isolates collected in the field during 2011 (Figure 22). At the time of the first rating, both parents equaled in susceptibility to Avocet. With subsequent ratings, disease severities on AC Domain and RL4452 were only slightly lower than was recorded on Avocet (Figure 22). There was no significant difference in DS detected between the two parents. Over the course of the experiment, no sign of a hypersensitive response was noted for either parent. Single isolate testing was also performed on the parents using several purified races derived from the race mixture. Similarly, these results showed that both parents were susceptible to all races tested (data not shown).

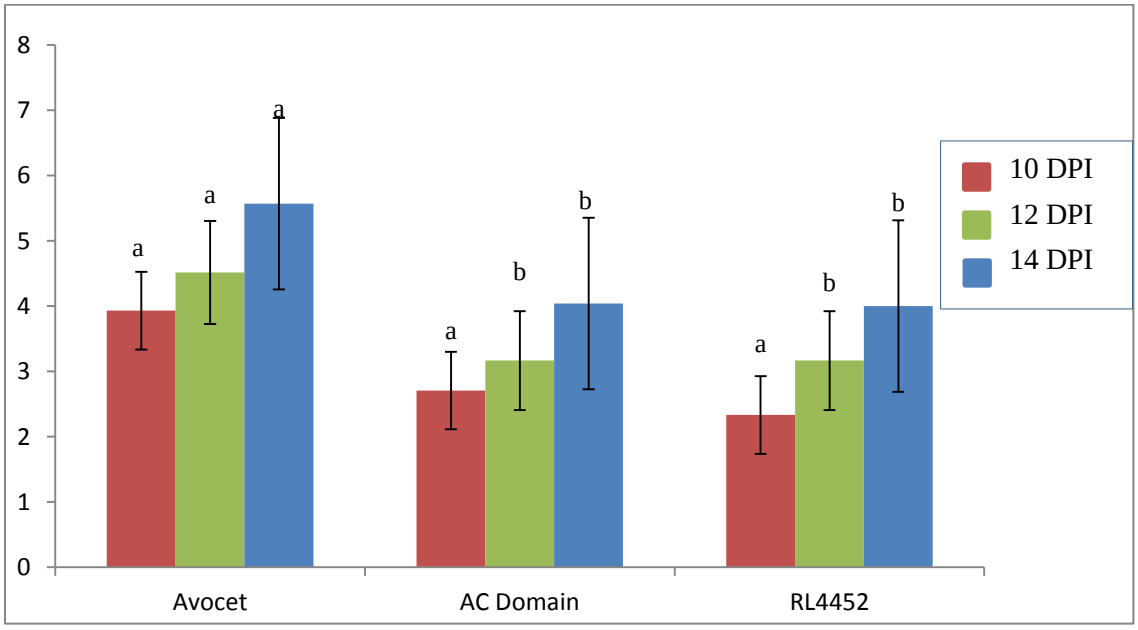

Figure 22: Stripe rust disease scores recorded on AC Domain, RL4452 and the susceptible control Avocet, at three different rating times in the phytotron experiment.

5.0 DISCUSSION

Breeding for durable resistance is critical for ensuring sustainable long-term protection against wheat yield losses caused by *P. triticina* and *P. striiformis* f.sp. *tritici*. One strategy to achieve durable resistance has been through deployment of "slow-rusting" APR genes, some of which have maintained their effectiveness for several decades (Kolmer et al. 2007). There are several general defining characteristics that distinguish APR from major gene resistance: 1) APR is race non-specific conferring resistance to all pathogen races, 2) APR is best expressed in the adult plant, and 3) APR confers only partial resistance characterized by a susceptible infection type (Roelfs et al. 1992). To date, three durable APR gene complexes have been identified in wheat, all of which confer broad spectrum APR to leaf and stripe rust. This is important for disease resistance breeding because multiple disease resistance can be obtained through the action of a single gene, making them valuable to breeders on a global scale. Therefore, there is a significant need to identify additional APR genes in wheat. The main goal of this research was to genetically localize the rust APR in AC Domain. Elucidating the nature of the rust resistance in AC Domain is important because the line has been commonly used as a parent in Canadian breeding programs, valued as a source of pre-harvest sprouting resistance (Townley-Smith and Czarnecki 2008b, McCallum et al. 2012a). To accomplish the listed objective, a mapping population derived from a cross between resistant parents RL4452/AC Domain was examined. A secondary objective was to utilize the mapping population to test for additive interactions between APR genes in conferring leaf and stripe rust resistance. Because the mapping population was shown to segregate for a putative APR gene on 2BS, in addition to *Lr34* and *Lr46* (Figure 4), it was possible to examine the genetic interaction between these loci.

5.1 Molecular Analysis and Parental Screening

AC Domain was previously thought to carry *Lr34* because it expresses field level APR similar to other known *Lr34* carriers (Liu and Kolmer, 1997), and because AC Domain amplifies the *Lr34* + allele at the *csLV34* (tightly linked to *Lr34*) locus (Lagudah et al. 2006). However, it was later shown that a rare recombination had occurred in AC Domain between *Lr34* and the *csLV34* locus, which resulted in the incorrect classification of AC Domain as an *Lr34* carrier (Lagudah et al. 2009). The strong field resistance displayed by AC Domain in five out of six

testing environments reported here (Table 4) supports the previous postulation that AC Domain carries an alternative APR gene.

When AC Domain was first registered in 1993 it was rated very resistant (VR) to leaf rust (Townley-Smith and Czarnecki 2008b), presumably due to the action of seedling genes *Lr10* and *Lr16* and at least one APR gene independent from *Lr34* (Liu and Kolmer 1997, McCartney et al. 2005b). Since its release, the field resistance in AC Domain has declined slightly, in part because *Lr10* is no longer effective to North American races of leaf rust (Liu and Kolmer 1997), and because *Lr16* has been at least partially defeated (McCartney et al. 2005a). In the present study, AC Domain usually expressed an effective level of leaf and stripe rust APR (Table 2). In most environments, DS of AC Domain was comparable, albeit slightly higher than DS of the Thatcher-*Lr34* check. These results were consistent with the ten-year performance of AC Domain in a permanent leaf rust nursery in Manitoba (Brent McCallum, personal communication). The only exception to this was at Lethbridge in 2011, where AC Domain expressed intermediate reaction to stripe rust. Because historical data on the stability of the stripe rust resistance in AC Domain was not previously available, further testing will be required to confirm the effectiveness of its stripe rust resistance across different environments. In light of the fact that AC Domain does not carry *Lr34* or *Lr46*, the field resistance expressed by AC Domain suggests the presence of unidentified/unknown R genes, which is especially apparent in the case of leaf rust.

Since AC Domain is a known carrier of *Lr16* (McCartney et al. 2005a,b), it was important to test for the potential effects of *Lr16* on phenotype. The mapping population was screened with the tightly linked primer *Xwmc764* which localized to its expected location on the distal end of chromosome 2BS (McCartney et al. 2005a,b; Figure 4). However, contrast analysis showed *Lr16* was only associated with a small reduction in leaf rust DS in combination with *Lr34*, *Lr46* and *Q.usw-2B1* (Figure 9), and its effect was only detected at only a single testing environment. The reduced effectiveness of *Lr16* at field level is consistent with recent studies (McCallum and Seto-Goh 2003, 2004). In Canada, the frequency of isolates virulent to *Lr16* has been increasing steadily over the past 15 years, in part due to the increased cultivation of CWRS cultivars such as AC Barrie that carry the gene (McCallum and Seto-Goh 2004). Recent race surveys have shown that in 2001, 74.1 % of isolates collected in a Canadian race survey were virulent to *Lr16*, up from only 35.5 % the previous year (McCallum and Seto-Goh 2003, 2004).

However, *Lr16* could still provide some level of protection in combination with other genes if a proportion of the pathogen population in a given location were to consist of an *Lr16* avirulent race. This could explain why the effect of *Lr16* was only detected in one testing environment in this study. Since other studies have shown that *Lr16* can work with *Lr34* to enhance leaf rust resistance (German and Kolmer 1992), the gene could still be considered an important component of the resistance in AC Domain, and other Canadian cultivars, including hexaploid wheat cultivars AC Karma, AC Majestic, AC Splendor, Columbus and Grandin (McCartney et al. 2005a).

Molecular results from this study confirmed the presence of *Lr34* in RL4452. Interestingly, RL4452 also amplified the *Lr46* (+) marker allele (similar to the check WPCH-01), and therefore likely contributed two of the APR genes segregating in mapping population. This finding was supported by three important observations. First, RL4452 was highly resistant to leaf and stripe rust across environments and consistently expressed a stronger level of resistance than the Thatcher-*Lr34* isogenic line. In addition, RL4452 was found to contribute resistance QTL for CI and AUDPC, both of which mapped close to the expected location of *Lr46* on chromosome 1B (Figure 4). Finally, analysis of the *Lr46* KASP marker in a diverse set of hexaploid wheat cultivars showed that several cultivars derived from Glenlea also carried the *Lr46* (+) marker allele (Appendix 5). However, since the *Lr46* KASP marker is not gene-specific it cannot be confirmed if this marker is completely diagnostic for *Lr46*. Several SSR markers known to flank the *Lr46* locus (*Xbarc80*, *Xwmc44*, *Xgwm140*; Figure 4) mapped to the *Lr46* genomic region on chromosome 1BL, and these results support the possibility that RL4452 may carry *Lr46*.

5.2 Linkage and QTL Mapping of *Lr34* Non-Carriers

Field results confirmed AC Domain expresses field level resistance that phenotypically resembles known lines carrying *Lr34*; therefore attempts were made to localize this resistance. *Lr34* was highly effective at conferring resistance to both leaf and stripe rust in the RL4452/AC Domain mapping population (Figure 2). Thus, it was likely that *Lr34* could be masking the phenotypic expression of additional resistance gene(s) segregating in the population. Indeed, others have shown that *Lr34* can mask the phenotypic expression of alternative resistance segregating in a mapping population (Martinez et al. 2001, Lillemo et al. 2008). To overcome

this, experimental lines were classified into *Lr34* carriers and non-carriers, and genetic mapping and QTL analysis was limited only to the *Lr34* non-carriers.

First, a high density genetic map was created using a 9K iSelect assay recently designed for wheat, and select SSR loci (Appendix 3). The genetic map spanned 3117 cM, which was similar to the length of the previous SSR linkage map (2793 cM) developed for this population (McCartney et al. 2006). The number of linkage groups in the high density map (35) was larger than the original map (27), which probably reflects the use of more stringent LOD thresholds for assigning markers to linkage groups in the present study. An issue with the current iSelect assay is that no prior map information is available for the SNP markers, so assigning linkage groups to specific chromosomes was a challenge. To overcome this, iSelect probe sequences were aligned using BLAST against the current Chinese Spring wheat survey sequence (developed by the International Wheat Genome Sequencing Consortium) to identify putative chromosomal locations for each probe. Several individual probes mapped to multiple chromosomes, so it was not always possible to assign those to an appropriate linkage group. However, in all cases it was possible to assign a putative chromosome assignment to linkage groups for which QTL were identified. SSR markers with known chromosomal positions were added to the genetic map to confirm correct assignment. The majority of the SSR marker data integrated well with the SNP data. The marker order and genetic distances of SSRs had a good correspondence with the SSR map previously published (McCartney et al. 2006) suggesting the newly developed map reported here is of good quality.

Results of the QTL mapping study confirmed that AC Domain was an important source of rust resistance. Of several major and minor QTL that were identified, all but three QTL (*QYr.usw-6B, QCis.usw-1B,* and *QAudpc.usw-1B)* were derived from AC Domain (Figure 4, Table 6). Two QTL (*Q.usw-2B1*, *QLrs.usw-2B2*) associated with leaf rust resistance mapped to chromosome 2BS, and resistance in both cases was derived from AC Domain. The most promising discovery was the detection of the major rust resistance QTL *Q.usw-2B1* (*QLrs.usw-2B1 QLrp.usw-2B1 QYr.usw-2B1 QCis.usw-2B1 QCip.usw-2B1, QAudpc.usw-2B1,* and *QLtn.usw-2B1)* that was detected across all environments (Figure 4, Table 6). *Q.usw-2B1* was associated with increased leaf and stripe rust APR, along with reduced AUDPC and CI scores. AUDPC is often used to measure APR (Roelfs et al. 1992). This is because cultivars that express APR have reduced AUDPC arising from an increased latent period, reduced infection frequency,

smaller uredinium size and reduced spore production (Ohm and Shaner 1976, Wilcoxson 1981, Herrera-Foessel et al. 2007). In addition, *Q.usw-2B1* was always associated with QTL for LTN. Given that *Q.usw-2B1* is associated with leaf and stripe rust resistance and mapped with a QTL for LTN (Figure 4), it appears to be acting in a manner similar to other reported APR genes of wheat (Dyck et al. 1966, Singh 1992, Lillemo et al. 2008, Singh et al. 1998, William et al. 2003, Hiebert et al. 2010, Herrera-Foessel et al. 2011). Additional evidence that supports *Q.usw-2B1* confers APR came with the seedling rust tests, which showed that AC Domain was MS-S to the same leaf rust races used to assess resistance in field studies (Table 8, Figures 19c, 20). AC Domain did express a mixture of compatible and incompatible ITs, which was expected since the line is known to carry the partially effective seedling resistance gene *Lr16* (McCartney et al. 2005a,b). The predominant IT recorded on AC Domain was consistent with a compatible reaction that closely resembled the phenotypic reaction recorded on the Thatcher-*Lr16* check. Even though there were obvious signs of a host response in both lines, the pustules were large, healthy and actively sporulating, which indicates that the host response was not fully effective. However, based on these results AC Domain cannot be classified as fully leaf rust susceptible at the seedling stage presumably due to the small effect of *Lr16*. The ineffectiveness of the leaf rust resistance at the seedling stage suggests that the resistance in AC Domain is most effectively expressed at the field level at the adult-plant stage. The stripe rust seedling testing was more conclusive since both parents were completely susceptible to the mixture of races, and there was no sign of any hypersensitive response in either parental line.

Several important rust resistance genes have been already been reported on chromosome 2BS (McIntosh 2008). These include *Yr5, Yr7, Yr27, Yr31, YrV23, YrSp, YrQz, YrTp1* and *YrCN19* (Luo et al. 2008). Previous studies have also identified QTL for rust APR on chromosome 2BS, but comparison between studies can often be difficult when different types of markers are used. Two studies have reported the major rust QTL *QYr.sgi-2B1* derived from the cultivar Kariega (Ramburan et al. 2004, Prins et al. 2011), both of which found *QYr.sgi-2B1* to be associated with the SSR marker *Xgwm148*, proximal to the marker *Xbarc200* (note that both SSR markers were also mapped in the present study, Figure 4). In a separate study, Carter et al. (2009) located the major QTL *QYrlo.wpg-2BS* associated with HTAP resistance to stripe rust in the spring wheat cultivar Louise, which spanned a similar marker interval between *Xwmc474-Xgwm148*. The proximity of *QYr.sgi-2B.1* to *QYrlo.wpg-2BS* could indicate the two loci are the

same. However, in the present study, *Q.usw-2B1* mapped approximately 45 cM distal to *Xgwm148* and is therefore not likely to be the same QTL.

In the present study, A second leaf rust QTL, *QLrs.usw-2B2*, mapped to chromosome 2BS but was only detected in one testing environment. *QLrs.usw-2B2* mapped approximately 46 cM distal to *QLrs.usw-2B1* and was associated with the *Lr16* diagnostic primer *Xwmc764* (McCartney et al. 2005a, b, 2006). Because of the relatively large distance between *QLrs.usw-2B1* and *QLrs.usw-2B2* (46 cM; Figure 4), they were classified as two independent QTL.

The 2011 Lethbridge stripe rust data revealed that in the absence of *Lr34*, a large proportion of experimental lines were resistant to the disease (Figure 2). In 2011, the bimodal distribution in the *Lr34* non-carriers suggested the population could be segregating for a single major resistance gene as indicated by the appearance of two predominant groups (i.e. resistant, susceptible). However, the data did not fit the expected Chi-Square 1:1 ratio (data not shown), as a number of lines displayed intermediate disease reaction between the two main groups. This was also reflected in the QTL results since instead of finding a single gene, three QTL with minor effects were detected. These included *QYr.usw-6B*, *QYr.usw-4A* and *QYr.usw-2B1*. These QTL were stable over both years of field testing, suggesting that all three loci are important components of the stripe rust resistance in the RL4452/AC Domain population.

The effectiveness of the stripe rust resistance expressed by AC Domain was variable. In 2011, AC Domain was rated as moderately susceptible to stripe rust, but in 2012 expressed an effective level of stripe rust APR (Table 4). *QYr.usw-2B1* explained a significant portion of the phenotypic variance in each year and is therefore an important component of the stripe rust resistance in AC Domain. In 2012, *QYr.usw-6B* (derived from RL4452) was the most important stripe rust resistance locus contributing to the disease resistance in the mapping population. The high temperature adult-plant (HTAP) resistance gene *Yr36* has previously been located on chromosome 6BS in wild emmer wheat (*T. turgidum* ssp. *dicoccoides*), however, *Yr36* is not present in most modern wheat varieties (Fu et al. 2009). Therefore *Yr36* is not likely to be associated with *QYr.usw-6B.*

5.3 Development of DNA Markers for a Novel APR on Chromosome 2BS

Given the discovery of *Q.usw-2B1,* and considering its large effect on all traits in the *Lr34* non-carriers, it was reasonable to assume that these effects could be extended to the *Lr34* carrying lines. Because SNP marker data was only available for the *Lr34* non-carriers, a new

approach was devised to convert iSelect SNP probe sequence information into PCR based markers, which could then be used to screen the *Lr34* carrying lines. These efforts resulted in the development of two new markers (*SSCPSNP-wsnp_Ra_rep_c117300_96881829*, *SNP-wsnp_Ex_c16144_24583060*, Appendix 4) that were associated with the peak of *Q.usw-2B1*. However, when applied to the diversity panel of hexaploid wheat cultivars, only *SSCPSNP-wsnp_Ra_rep_c117300_96881829* was informative. Because the results only represent the marker allele for the *Q.usw-2B1*, further testing will be required in a much larger panel, coupled with detailed phenotyping experiments, to determine if a historical recombination between the proposed gene and the marker has occurred in some lines or genetic backgrounds. In addition, more work will be required to develop markers that are more robust and suitable for high-throughput MAS applications. The discrepancies between the iSelect and KASP genotypic results for the marker *SNP-wsnp_Ex_c16144_24583060* may have been due to copy number variation in the diversity panel causing ascertainment bias at that locus.

5.4 Evidence of Additive Gene Interaction, and Implications towards Breeding for Durable Rust Resistance in Wheat

The rust resistance QTL *Q.usw-2B1* should be considered by breeders aiming to develop durable new varieties with superior rust resistance. *Q.usw-2B1* shares many desirable characteristics with the other durable APR genes previously described in wheat, including the "slow rusting" phenotype characterized by a susceptible infection type associated with reduced DS and AUDPC. In the present study, lines carrying *Q.usw-2B1* often expressed effective dual pathogen (*P. triticina*, *P. striiformis*) APR. However, reliance on *Q.usw-2B1* when used as the sole resistance gene is not advised since it did not always confer an effective level of resistance in all testing environments. Some studies have shown that stacking multiple APR genes in a cultivar can result in near immunity to rust in the field (Singh et al. 2000a). To test this hypothesis, the interactions between known resistance loci segregating in the mapping population were investigated.

Results showed that the interaction between *Lr34* and *Q.usw-2B1* was significant for three out of four leaf rust environments (Appendix 6, 7). The only exception was at Portage 2012, where the resistance conferred by *Q.usw-2B1* was less effective under much higher disease pressure. At Saskatoon 2012, the interaction between *Lr34* and *Q.usw-2B1* was additive, and the

presence of both positive alleles resulted in a superior level of resistance that was not achievable with the action of either gene on its own (Figure 10). In this environment, disease conditions were optimal for the expression of *Q.usw-2B1*. However, in most environments the effect of *Lr34* masked the expression of *Q.usw-2B1* (Figures 9, 13).

At Saskatoon 2011, there was a significant four way interaction among *Lr34, Lr46, Lr16* and *Q.usw-2B1* (Appendix 6). Results clearly showed that *Lr34* had the strongest effect towards decreasing DS, although a general trend of decreasing DS was observed with the accumulation of positive alleles across all four loci (Figure 9). For many allelic combinations, DS was well below the average of the Thatcher-*Lr34* check. At Saskatoon in 2011, the additive resistance conferred by positive alleles for *Lr46*, *Lr16* and *Q.usw-2B1* in the absence of *Lr34* resulted in a high level of resistance that was equally as effective as the resistance conferred by *Lr34* alone. Taken together, these results show that *Lr34* is clearly the most important and stable genetic factor influencing leaf rust and associated traits, but in some environments the additive gene action of *Lr34* and *Q.usw-2B1* can result in superior resistance. This could explain why some experimental lines showed transgressive segregation for leaf rust resistance, and were more resistant than the Thatcher-*Lr34* isogenic line (Table 2, Appendix 2).

Because *Lr34* and *Lr46* both confer a slow-rusting phenotype against leaf and stripe rust, it was initially thought that their action could work in an additive manner to provide an increased level of rust resistance in the field. Although molecular evidence does suggest that *Lr46* is segregating in this population (Figure 3), results showed the *Lr46* marker allele was having only a small (non-additive) effect when combined with *Lr34* (data not shown). Furthermore, the interaction between *Lr34* and *Lr46* was not significant across environments, and the main effect of *Lr46* was only significant in some environments (Appendix 6-8). These findings are consistent with several studies (Singh et al. 1998, Martinez et al. 2001, Lillemo et al. 2008). One consideration is that *Lr46* might not provide enough resistance on its own under high disease pressure (Singh et al. 1998). Martinez et al. (2001) compared isogenic lines of the susceptible cultivar Lalbahadur and found that the resistance conferred by *Lr46* was notably less than that conferred by *Lr34*. There are conflicting reports as to the effectiveness of *Lr46* on leaf and stripe rust resistance (William et al. 2003, 2006, Lillemo et al. 2008). In a similar study, Lillemo et al. (2008) observed a non-additive effect of *Lr34* and *Lr46* in a bread wheat population derived from a cross between Avocet-*Yra* and Saar, which the authors attribute to an overlap in the

resistance mechanisms of these two genes. One important consideration is that the expression of *Lr46* is highly sensitive to genetic background (Brent McCallum, personal communication). In the present study, *Lr34* exhibited a highly resistant response to both diseases, in many cases masking expression of any other genes. In addition, results showed that *Lr34* was always associated with reduced CI and AUDPC scores. This is consistent with reports that have shown *Lr34* is responsible for an increase in latent period, a reduction in the number of haustoria that are formed and an increase in early sporeling abortion (Rubiales and Niks 2005). The high correlations between AUDPC, CI and DS indicate all three ratings may be useful in quantifying adult-plant resistance. This finding was expected since low CI and AUDPC scores are almost always associated with low DS (Roelfs et al. 1992). However, because AUDPC requires significantly more time and labor input due to multiple ratings, these results suggest that a single rating may be sufficient to adequately measure DS.

Statistical analysis of the Lethbridge data did not detect the effect of *Q.usw-2B1* on stripe rust DS. This was also reflected by the low R^2 values in the QTL analysis. The fact that statistical analysis failed to detect *Q.usw-2B1* for stripe rust in the overall population can probably be at least partly attributed to the confounding effects of *Lr34*, but might also reflect some of the inherent difficulties in detecting APR genes with small effects. Another important consideration is that *Q.usw-2B1* was not the most important stripe rust QTL identified in this study. In fact, *QYr.usw-6B*, derived from RL4452, explained more of the stripe rust variation in both years. In addition, a third QTL, *QYr.usw-4A*, also had minor effects against stripe rust, and that QTL was detected in both years. However, attempts to successfully develop working markers for *QYr.usw-6B* and *QYr.usw-4A* failed. More work will be required to develop co-segregating markers diagnostic for these important rust resistance loci.

Some important insights can be drawn from the molecular analysis of the diversity panel regarding the status of three common resistance loci *Lr34, Lr46* and *Q.usw-2B1* in Canadian germplasm (Appendix 5). Breeding efforts have in large part been successful in avoiding reliance on single genes as results showed that many of the lines in the diversity panel carry at least two of these three resistance genes. For example, CDC Kernen, NRG10, Conquer and Muchmore carried resistance alleles at *Lr34, Lr46* and *Q.usw-2B1* (Appendix 5). In contrast, 15 of the lines from the diversity panel scored here carried negative alleles at all three loci (Appendix 5). It appears that *Q.usw-2B1* is already quite common in Canadian germplasm. The locus is

commonly found in lines derived from a Neepawa or Roblin background, such as AC Splendor, AC Intrepid and Goodeve. Despite being the most effective of the APR genes in this study, *Lr34* is only carried by 35% of the diversity panel lines tested (Appendix 5). Results suggest *Lr46* is also common in Canadian germplasm and is carried by 45% of lines in the diversity panel (Appendix 5).

Despite being used frequently as a parent in Canadian breeding programs, marker analysis suggests AC Domain did not pass *Q.usw-2B1* on to several of its progeny (Figure 6). These lines include Kane, AC Superb and Stettler. At the time they were released, Kane and AC Superb were both highly resistant to the prevalent races of *P. triticina* in western Canada (Fox et al. 2007b, Townley-Smith et al. 2010), in contrast to Stettler which was moderately susceptible (DePauw et al. 2009b). Interestingly, Kane, AC Superb and Stettler also do not carry positive marker alleles for *Lr34* or *Lr46* (Appendix 5) suggesting these lines might carry additional resistance genes. Kane is resistant to the most prevalent races of *P. triticina*, and carries *Lr21* derived from McKenzie, and *Lr16* derived from either McKenzie or AC Domain (Fox et al. 2007b). Also derived from AC Domain, the spring wheat cultivar Waskada carries only *Q.usw-2B1* but expresses moderate resistance to *P. triticina* (Fox et al. 2009). Muchmore probably inherited *Q.usw-2B1* from AC Domain, but is also a carrier of *Lr34* and *Lr46*, which it inherited from Alsen (Appendix 5), and thus has very strong resistance to *P. triticina* at field level (Depauw et al. 2011b). *Q.usw-2B1* is not common in cultivars derived from Glenlea, including Glencross, Burnside, CDC Rama and CDN Bison. However, these lines do carry the positive marker alleles for *Lr34* and *Lr46*, and therefore probably have effective levels of leaf rust resistance (Appendix 5).

To complement *Q.usw-2B1*, breeding strategies should focus on implementing stacked resistance packages combining *Lr34*, *Q.usw-2B1* and other genes to ensure effective disease resistance under a diverse range of environments. Furthermore, if *Q.usw-2B1* functions as an APR gene, it should be combined with other all-stage resistance genes to ensure plants are not vulnerable at the seedling stage. It has been suggested that combining resistance genes with different resistance mechanisms, such as race non-specific APR with race-specific resistance could result in enhanced resistance, and increased durability in the field (Rubiales and Niks 2005). The molecular marker *SSCPSNP-wsnp_Ra_rep_c117300_96881829* reported here should help breeders ensure *Q.usw-2B1* is being transferred into progeny during crossing and should be

useful to develop elite cultivars with stacked resistance genes. However, more work will be required to develop a more user-friendly marker.

5.5 Final Conclusions

The main objectives of this study were to genetically localize the rust APR in AC Domain, and to study the genetic effects of multiple rust resistance loci. The following conclusions were made based on the data collected:

1. AC Domain carries the major rust resistance QTL *Q.usw-2B1,* which shares similar characteristics to other reported APR genes described in wheat:

- *Q.usw-2B1* is associated with dual pathogen resistance to both leaf and stripe rust;
- *Q.usw-2B1* confers a partial resistance at field level which phenotypically resembles the "slow-rusting" phenotype associated with reduced DS and AUDPC;
- *Q.usw-2B1* is associated with increased LTN severity;
- AC Domain, the donor of *Q.usw-2B1,* was susceptible at the seedling stage to both leaf and stripe rust.

Taken together, the evidence suggests one of two possibilities: This locus could contain an APR gene under pleiotropic control, or alternatively, contains two separate, but linked, major genes conferring resistance. The evidence suggests the former, meaning *Q.usw-2B1* could be a novel APR gene analogous to other APR genes like *Lr34/Yr18, Lr46/Yr29* and *Lr67/Yr46* that have previously been described in wheat (Dyck 1987, Singh et al. 1998, Hiebert et al. 2010).

2. AC Domain does not carry the APR gene *Lr46,* but instead RL4452 appears to be a carrier. *Lr46* had only a minor effect on the expression of leaf and stripe rust resistance in the mapping population, which was not always detectable. *Lr46* did not provide an effective level of resistance on its own, and did not enhance disease resistance in the majority of gene combinations. In contrast, the effects of *Lr34* were consistent across environments, and always provided a high level of resistance making it the most effective gene in the RL4452/AC Domain population. In most cases, *Lr34* masked the expression of other resistance loci. Results from this

study have shown that traits associated with *Lr34* are more strongly expressed than other APR genes such as *Lr46*, and *Q.usw-2B1*.

3. AC Domain is moderately susceptible/susceptible to leaf and stripe rust at the seedling stage. Although AC Domain does carry the seedling gene *Lr10*, western Canadian races of rust have gained virulence to it. AC Domain also carries the partially defeated seedling gene *Lr16*, which does provide some level of leaf rust resistance to western Canadian isolates. However, the effect of *Lr16* was not consistently detected in the present study, and even when detected was only responsible for a small decrease in DS. Furthermore, it is unlikely that *Lr16* could confer a high level of resistance on its own based on the extreme susceptibility of the Thatcher-*Lr16* check to both diseases over the course of the study, and the inconsistent detection of the gene through QTL analysis. The "mixed reaction" infection type recorded on AC Domain at the seedling stage is presumed to be because of the effects of *Lr16* in eliciting some level of hypersensitive reaction to leaf rust.

4. In some environments, *Q.usw-2B1* can work additively with *Lr34* to provide a superior level of rust resistance, and can even achieve the same level of resistance provided by *Lr34*.

5.6 Future Work

- There are multiple rust resistance genes segregating in the RL4452/AC Domain population. To more accurately study the effects of *Q.usw-2B1*, attempts should be made to transfer the gene into a susceptible genetic background, and then develop a bi-parental mapping population. This would allow for more precise measurement of the genetic effects of the locus on rust resistance, and could yield a more precise map location for *Q.usw-2B1*. One additional benefit would be the ability to perform seedling testing without the confounding effects of additional resistance genes to confirm that the locus is only effective at the adult–plant stage.
- Results from this study have shown the resistance provided by *Q.usw-2B1* can be variable across environments, especially with respect to stripe rust. More work will be required to evaluate the effectiveness of *Q.usw-2B1* in more stripe rust environments to fully characterize its effectiveness as a stripe rust APR gene. Furthermore, more work will be required to develop working PCR-based markers for *QYr.usw-6B* and *QYr.usw-4A*, which

likely play an important role in conferring disease resistance in the AC Domain/RL4452 population.

- APR genes in wheat can be sensitive to genetic background. Efforts should be made to examine potential effects of *Q.usw-2B1* in different genetic backgrounds to see if there are significant differences, as is often the case with *Lr46*.
- The genetic map and newly developed molecular markers for *Q.usw-2B1* reported here represent a good starting point for future genetic research. However, the best working marker (*SSCPSNP-wsnp_Ra_rep_c117300_9688182)* for *Q.usw-2B1* is not user friendly, because of the laborious nature of running SSCP gels, and is thus not suitable for high-throughput use in MAS breeding programs. On the other hand, the KASP marker that was developed worked very well in the mapping population, but was not informative when tested on a diverse set of lines. With new 90K SNP technology, it should be possible to identify additional co-segregating markers with *Q.usw-2B1*, and thus additional candidates for conversion to robust KASP markers suitable for use in marker assisted selection. Future work will also reveal whether historical recombination between the *SSCPSNP-wsnp_Ra_rep_c117300_9688182* marker and the proposed gene associated with *Q.usw-2B1* has occurred in some lines causing incorrect classification.
- *Lr34* is known to encode an ABC transporter protein, but it is unknown whether the other APR genes encode similar proteins. With the ever increasing availability of wheat genomic sequence information, sequence analysis should be performed using the *Lr34* transporter sequence targeted to the known chromosomal locations of the other APR genes, such as *Q.usw-2B1, Lr46* and *Lr67*. The increasing reliance on APR genes to confer resistance makes it important to fully understand their mechanisms of resistance.

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7.0 APPENDICES

Appendix 1. Variance estimates for random effects and F-values for fixed effects from analysis of variance (ANOVA) of percent disease severity (DS) at leaf rust testing environments at Saskatoon and Portage, and the stripe rust environments at Lethbridge. Data was collected over 2011-2012 field seasons and was analyzed separately by year.

	Saskatoon		**Portage**		**Lethbridge**	
	2011	**2012**	**2011**	**2012**	**2011**[a]	**2012**
			Random Effect Variance Estimates			
Block(Rep)[b]	0.289	0	na	na	na	na
Rep	7.457	0.139	61.337	4.808	na	2.456
Residual	37.083***	92.491***	129.88***	137.730***	na	67.185***
			Fixed Effect F-Values			
Entry	12.80***	9.29***	3.68***	16.84***	na	19.53***

*P<0.05, **P<0.01, ***P<0.001

[a]Note that 2011 Lethbridge stripe rust data was not replicated, and ANOVA was not performed.

[b]The Block(Rep) random effect was only measured at Saskatoon testing environments.

Appendix 2. Least-square means (LS means) for percent disease severity (DS) ratings taken from lines in the RL4452/AC Domain mapping population. Ratings were taken in leaf rust testing environments at Saskatoon and Portage, and the stripe rust environments at Lethbridge. Data was collected over 2011-2012 field seasons and was analyzed separately by year. All LS means estimates are given with their standard errors (SE).

	Saskatoon				Portage				Lethbridge		
	2011		2012		2011		2012		2011[a]	2012	
ID	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	Estimate	SE
93E54*A10	0.690	3.865	10.000	5.557	0.333	7.984	18.333	6.893	0.000	15.000	4.818
93E54*A103	13.394	3.865	25.000	5.557	16.667	7.984	50.000	6.893	0.000	20.000	4.818
93E54*A104	36.658	3.865	40.000	5.557	37.823	9.249	60.000	6.893	65.000	68.333	4.818
93E54*A11	2.338	3.865	5.000	5.557	2.000	7.984	20.000	6.893	0.000	15.000	4.818
93E54*A13	46.767	3.865	18.333	5.557	46.667	7.984	80.000	6.893	75.000	80.000	4.818
93E54*A16	0.681	3.865	3.667	5.557	0.333	7.984	0.667	6.893	5.000	15.000	4.818
93E54*A19	8.178	3.865	20.000	5.557	13.333	7.984	10.000	6.893	0.000	18.333	4.818
93E54*A26	1.017	3.865	11.667	5.557	3.667	7.984	13.333	6.893	0.000	16.667	4.818
93E54*A33	6.600	3.865	8.333	5.557	13.323	9.249	7.000	6.893	0.000	13.333	4.818
93E54*A35	1.017	3.865	2.333	5.557	20.000	7.984	33.333	6.893	0.000	15.000	4.818
93E54*A40	3.694	3.865	10.000	5.557	31.008	12.282	71.667	6.893	0.000	20.000	4.818
93E54*A46	0.703	3.865	3.667	5.557	5.000	7.984	46.667	6.893	0.000	11.667	4.818

93E54*A48	5.280	3.865	33.333	5.557	20.181	9.248	3.667	6.893	0.000	10.000	4.818
93E54*A5	10.095	3.865	8.333	5.557	30.181	9.248	75.000	6.893	0.000	15.000	4.818
93E54*A53	5.334	3.865	8.333	5.557	11.667	7.984	8.333	6.893	0.000	16.667	4.818
93E54*A59	3.583	3.865	5.000	5.557	1.000	7.984	2.333	6.893	0.000	20.000	4.818
93E54*A60	5.077	3.865	25.000	5.557	10.333	7.984	13.333	6.893	0.000	10.000	4.818
93E54*A65	6.732	3.865	16.667	5.557	15.823	9.249	29.084	8.401	0.000	-	-
93E54*A69	26.619	3.865	25.000	5.557	10.323	9.249	71.667	6.893	-	28.333	4.818
93E54*A72	8.435	3.865	16.667	5.557	11.667	7.984	13.333	6.893	0.000	23.333	4.818
93E54*A73	40.106	3.865	56.667	5.557	35.323	9.249	80.000	6.893	65.000	68.333	4.818
93E54*A76	26.653	3.865	35.000	5.557	38.333	7.984	71.667	6.893	65.000	83.333	4.818
93E54*A77	6.670	3.865	35.000	5.557	21.667	7.984	21.667	6.893	5.000	36.667	4.818
93E54*A81	13.332	3.865	36.667	5.557	23.333	7.984	55.000	6.893	65.000	38.333	4.818
93E54*A87	2.311	3.865	5.333	5.557	2.000	7.984	10.000	6.893	0.000	16.667	4.818
93E54*A88	4.943	3.865	18.667	5.557	15.333	7.984	3.667	6.893	0.000	15.000	4.818
93E54*A91	8.414	3.865	15.000	5.557	17.000	7.984	4.667	6.893	5.000	23.333	4.818
93E54*A92	3.715	3.865	18.333	5.557	13.323	9.249	23.333	6.893	0.000	55.000	4.818
93E54*A97	20.062	3.865	18.333	5.557	41.667	7.984	76.667	6.893	75.000	85.000	4.818
93E54*A99	10.002	3.865	15.000	5.557	18.667	7.984	73.333	6.893	0.000	23.081	5.871

93E54*B10	8.306	3.865	25.000	5.557	11.667	7.984	45.000	6.893	0.000	18.333	4.818
93E54*B11	26.689	3.865	36.667	5.557	51.667	7.984	80.000	6.893	65.000	71.667	4.818
93E54*B12	39.895	3.865	45.000	5.557	26.667	7.984	66.667	6.893	45.000	45.000	4.818
93E54*B15	3.548	3.865	2.333	5.557	7.000	7.984	61.667	6.893	0.000	26.667	4.818
93E54*B16	9.890	3.865	6.667	5.557	23.667	7.984	56.667	6.893	0.000	16.637	8.260
93E54*B19	19.927	3.865	35.000	5.557	31.667	7.984	80.000	6.893	65.000	67.738	5.871
93E54*B28	7.036	3.865	11.667	5.557	4.000	7.984	68.333	6.893	5.000	20.000	4.818
93E54*B33	3.670	3.865	5.333	5.557	17.681	9.248	0.333	6.893	65.000	13.333	4.818
93E54*B37	11.617	3.865	13.333	5.557	3.323	9.249	15.000	6.893	0.000	50.000	4.818
93E54*B4	36.634	3.865	53.333	5.557	35.000	7.984	43.333	6.893	45.000	21.667	4.818
93E54*B41	2.555	3.865	23.333	5.557	-1.177	9.249	10.000	6.893	0.000	61.667	4.818
93E54*B43	23.349	3.865	21.667	5.557	26.667	7.984	51.667	6.893	75.000	81.667	4.818
93E54*B44	18.390	3.865	26.667	5.557	14.000	7.984	35.000	6.893	0.000	23.333	4.818
93E54*B45	36.587	3.865	46.667	5.557	36.667	7.984	76.667	6.893	25.000	28.333	4.818
93E54*B47	3.717	3.865	13.333	5.557	15.323	9.249	1.667	6.893	0.000	20.000	4.818
93E54*B55	3.767	3.865	20.000	5.557	30.323	9.249	16.667	6.893	0.000	15.000	4.818
93E54*B61	33.316	3.865	70.000	5.557	41.667	7.984	75.000	6.893	75.000	58.333	4.818
93E54*B65	5.271	3.865	16.667	5.557	15.333	7.984	16.667	6.893	0.000	31.667	4.818

93E54*B68	16.657	3.865	36.667	5.557	30.323	9.249	71.667	6.893	-		43.333	4.818
93E54*B69	8.305	3.865	6.667	5.557	10.667	7.984	48.333	6.893		65.000	85.000	4.818
93E54*B74	3.627	3.865	8.667	5.557	5.333	7.984	3.667	6.893		0.000	28.333	4.818
93E54*B9	4.931	3.865	11.667	5.557	8.667	7.984	25.000	6.893		0.000	16.667	4.818
93E54*C10	3.610	3.865	6.667	5.557	12.681	9.248	20.000	6.893		0.000	13.333	4.818
93E54*C12	7.402	3.865	5.000	5.557	25.000	7.984	36.667	6.893		0.000	25.000	4.818
93E54*C16	5.422	3.865	3.667	5.557	-1.677	9.249	13.333	6.893		0.000	26.667	4.818
93E54*C19	0.073	3.865	0.667	5.557	4.000	7.984	11.667	6.893		0.000	20.000	4.818
93E54*C2	10.105	3.865	35.000	5.557	16.667	7.984	7.667	6.893		0.000	21.667	4.818
93E54*C21	23.365	3.865	28.333	5.557	32.823	9.249	58.333	6.893		85.000	83.333	4.818
93E54*C22	0.592	3.865	2.333	5.557	1.823	9.249	2.000	6.893		0.000	20.000	4.818
93E54*C23	16.529	3.865	23.333	5.557	10.667	7.984	13.333	6.893		0.000	28.333	4.818
93E54*C25	6.611	3.865	8.333	5.557	-7.992	12.282	30.000	6.893		0.000	15.000	4.818
93E54*C3	36.624	3.865	63.333	5.557	36.667	7.984	68.333	6.893		75.000	80.000	4.818
93E54*C30	6.720	3.865	15.000	5.557	12.000	7.984	58.333	6.893		45.000	15.000	4.818
93E54*C31	7.000	3.865	16.667	5.557	5.333	7.984	11.667	6.893		5.000	25.000	4.818
93E54*C33	11.685	3.865	33.333	5.557	33.333	7.984	51.667	6.893		5.000	20.000	4.818
93E54*C37	2.031	3.865	3.333	5.557	8.667	7.984	3.667	6.893		0.000	16.667	4.818

93E54*C38	30.072	3.865	25.000	5.557	33.333	7.984	80.000	6.893	75.000	63.333	4.818
93E54*C39	13.211	3.865	20.000	5.557	5.333	7.984	13.333	6.893	65.000	31.667	4.818
93E54*C41	3.658	3.865	13.333	5.557	14.000	7.984	9.333	6.893	5.000	20.000	4.818
93E54*C45	4.985	3.865	13.333	5.557	23.333	7.984	15.000	6.893	0.000	15.000	4.818
93E54*C47	4.914	3.865	15.000	5.557	2.681	9.248	21.667	6.893	45.000	16.667	4.818
93E54*C50	16.711	3.865	21.667	5.557	35.000	7.984	53.333	6.893	75.000	33.333	4.818
93E54*C53	6.695	3.865	10.333	5.557	12.681	9.248	36.667	6.893	0.000	18.333	4.818
93E54*C58	16.592	3.865	15.000	5.557	15.000	7.984	73.333	6.893	65.000	33.333	4.818
93E54*C6	46.823	3.865	70.000	5.557	47.823	9.249	80.000	6.893	25.000	55.000	4.818
93E54*C64	0.931	3.865	0.333	5.557	2.333	7.984	6.667	6.893	0.000	20.000	4.818
93E54*C69	5.498	3.865	3.333	5.557	18.333	7.984	35.000	6.893	5.000	16.667	4.818
93E54*C8	29.953	3.865	43.333	5.557	31.667	7.984	80.000	6.893	65.000	41.667	4.818
93E54*D10	1.055	3.865	3.667	5.557	5.681	9.248	4.000	6.893	0.000	15.000	4.818
93E54*D15	19.956	3.865	20.000	5.557	32.681	9.248	55.000	6.893	65.000	68.333	4.818
93E54*D17	20.073	3.865	23.333	5.557	21.667	7.984	61.667	6.893	65.000	45.000	4.818
93E54*D18	11.614	3.865	6.667	5.557	21.667	7.984	53.333	6.893	5.000	40.000	4.818
93E54*D20	49.954	3.865	66.667	5.557	30.323	9.249	76.667	6.893	25.000	78.333	4.818
93E54*D24	13.311	3.865	33.333	5.557	20.000	7.984	36.667	6.893	0.000	15.000	4.818

93E54*D26	8.291	3.865	15.000	5.557	8.323	9.249	21.667	6.893	0.000	35.238	5.871
93E54*D30	11.723	3.865	18.333	5.557	27.681	9.248	49.818	8.401	5.000	28.333	4.818
93E54*D33	3.624	3.865	11.667	5.557	3.667	7.984	5.333	6.893	5.000	26.667	4.818
93E54*D39	16.497	3.865	18.333	5.557	28.333	7.984	63.333	6.893	0.000	25.000	4.818
93E54*D45	16.627	3.865	13.333	5.557	5.333	7.984	53.333	6.893	0.000	21.667	4.818
93E54*D47	16.672	3.865	16.667	5.557	30.000	7.984	76.667	6.893	0.000	30.000	4.818
93E54*D5	3.644	3.865	1.000	5.557	2.333	7.984	10.000	6.893	0.000	23.333	4.818
93E54*D50	13.346	3.865	15.000	5.557	12.000	7.984	23.333	6.893	0.000	15.000	4.818
93E54*D51	19.988	3.865	28.333	5.557	32.681	9.248	63.333	6.893	5.000	16.667	4.818
93E54*D55	9.956	3.865	15.000	5.557	6.667	7.984	23.333	6.893	0.000	13.333	4.818
93E54*D58	5.318	3.865	6.667	5.557	2.000	7.984	15.000	6.893	5.000	16.667	4.818
93E54*D6	8.319	3.865	10.000	5.557	15.000	7.984	10.000	6.893	-	15.000	4.818
93E54*D9	7.077	3.865	8.333	5.557	12.000	7.984	10.333	6.893	0.000	13.333	4.818
93E54*E13	23.333	3.865	16.667	5.557	30.000	7.984	43.333	6.893	5.000	16.667	4.818
93E54*E14	8.813	3.865	7.000	5.557	14.000	7.984	50.000	6.893	65.000	21.667	4.818
93E54*E22	43.509	3.865	56.667	5.557	26.667	7.984	80.000	6.893	75.000	66.681	5.871
93E54*E23	33.383	3.865	43.333	5.557	40.000	7.984	70.000	6.893	25.000	15.000	4.818
93E54*E24	33.361	3.865	20.000	5.557	41.008	12.282	63.333	6.893	75.000	86.667	4.818

93E54*E35	2.294	3.865	3.333	5.557	0.667	7.984	2.000	6.893	75.000	31.667	4.818
93E54*E5	26.581	3.865	21.667	5.557	23.333	7.984	53.333	6.893	0.000	14.181	5.871
93E54*F1	26.657	3.865	50.000	5.557	20.000	7.984	76.667	6.893	65.000	51.667	4.818
93E54*F13	15.063	3.865	16.667	5.557	43.333	7.984	70.000	6.893	25.000	33.333	4.818
93E54*F20	13.207	3.865	25.000	5.557	16.667	7.984	20.000	6.893	0.000	15.238	5.871
93E54*F22	2.385	3.865	10.000	5.557	0.667	7.984	6.667	6.893	0.000	16.667	4.818
93E54*F29	50.057	3.865	36.667	5.557	40.323	9.249	76.667	6.893	75.000	19.181	5.871
93E54*F36	19.979	3.865	20.000	5.557	32.681	9.248	65.000	6.893	45.000	20.000	4.818
93E54*F38a	0.311	3.865	0.333	5.557	1.000	7.984	20.000	6.893	0.000	26.667	4.818
93E54*F38b	2.355	3.865	2.333	5.557	4.000	7.984	3.667	6.893	45.000	25.000	4.818
93E54*F39	10.025	3.865	6.667	5.557	36.667	7.984	-	-	25.000	31.667	4.818
93E54*F43	10.029	3.865	16.667	5.557	15.323	9.249	18.333	6.893	5.000	35.000	4.818
93E54*F44	9.877	3.865	15.000	5.557	35.181	9.248	76.667	6.893	5.000	33.333	4.818
93E54*F45	33.326	3.865	41.667	5.557	25.323	9.249	63.333	6.893	65.000	75.000	4.818
93E54*F47	30.025	3.865	31.667	5.557	42.823	9.249	61.667	6.893	5.000	26.667	4.818
93E54*F48	0.953	3.865	1.000	5.557	7.000	7.984	21.667	6.893	5.000	15.000	4.818
93E54*F49	16.764	3.865	28.333	5.557	27.823	9.249	76.667	6.893	75.000	36.667	4.818
93E54*F50	0.653	3.865	3.667	5.557	5.333	7.984	2.333	6.893	5.000	11.667	4.818

93E54*F57	14.982	3.865	21.667	5.557	21.667	7.984	61.667	6.893	5.000	15.000	4.818
93E54*F59	13.336	3.865	28.333	5.557	13.667	7.984	28.333	6.893	0.000	20.000	4.818
93E54*F6	16.704	3.865	23.333	5.557	33.333	7.984	73.333	6.893	15.000	46.667	4.818
93E54*F62	2.338	3.865	5.000	5.557	10.667	7.984	26.667	6.893	0.000	21.667	4.818
93E54*F65	3.659	3.865	8.333	5.557	0.667	7.984	25.000	6.893	5.000	15.000	4.818
93E54*F68	20.104	3.865	16.667	5.557	15.333	7.984	80.000	6.893	65.000	48.333	4.818
93E54*F8	8.405	3.865	11.667	5.557	22.681	9.248	63.333	6.893	0.000	23.333	4.818
93E54*F82	8.458	3.865	21.667	5.557	21.667	7.984	50.000	6.893	0.000	16.667	4.818
93E54*F87	4.895	3.865	8.333	5.557	1.000	7.984	5.000	6.893	5.000	15.000	4.818
93E54*G13	4.974	3.865	10.000	5.557	5.333	7.984	3.667	6.893	0.000	15.000	4.818
93E54*G17	16.619	3.865	13.333	5.557	16.667	7.984	70.000	6.893	0.000	51.667	4.818
93E54*G19	16.579	3.865	8.333	5.557	20.323	9.249	31.667	6.893	75.000	80.000	4.818
93E54*G3	6.691	3.865	10.000	5.557	15.000	7.984	55.000	6.893	65.000	36.667	4.818
93E54*G30	40.099	3.865	40.000	5.557	45.000	7.984	76.667	6.893	65.000	35.000	4.818
93E54*G32	19.923	3.865	30.000	5.557	15.333	7.984	16.667	6.893	0.000	15.000	4.818
93E54*G36	36.639	3.865	53.333	5.557	43.333	7.984	80.000	6.893	65.000	35.000	4.818
93E54*G4	36.719	3.865	43.333	5.557	35.323	9.249	80.000	6.893	75.000	80.000	4.818
93E54*G41	2.278	3.865	11.667	5.557	1.000	7.984	3.667	6.893	5.000	28.333	4.818

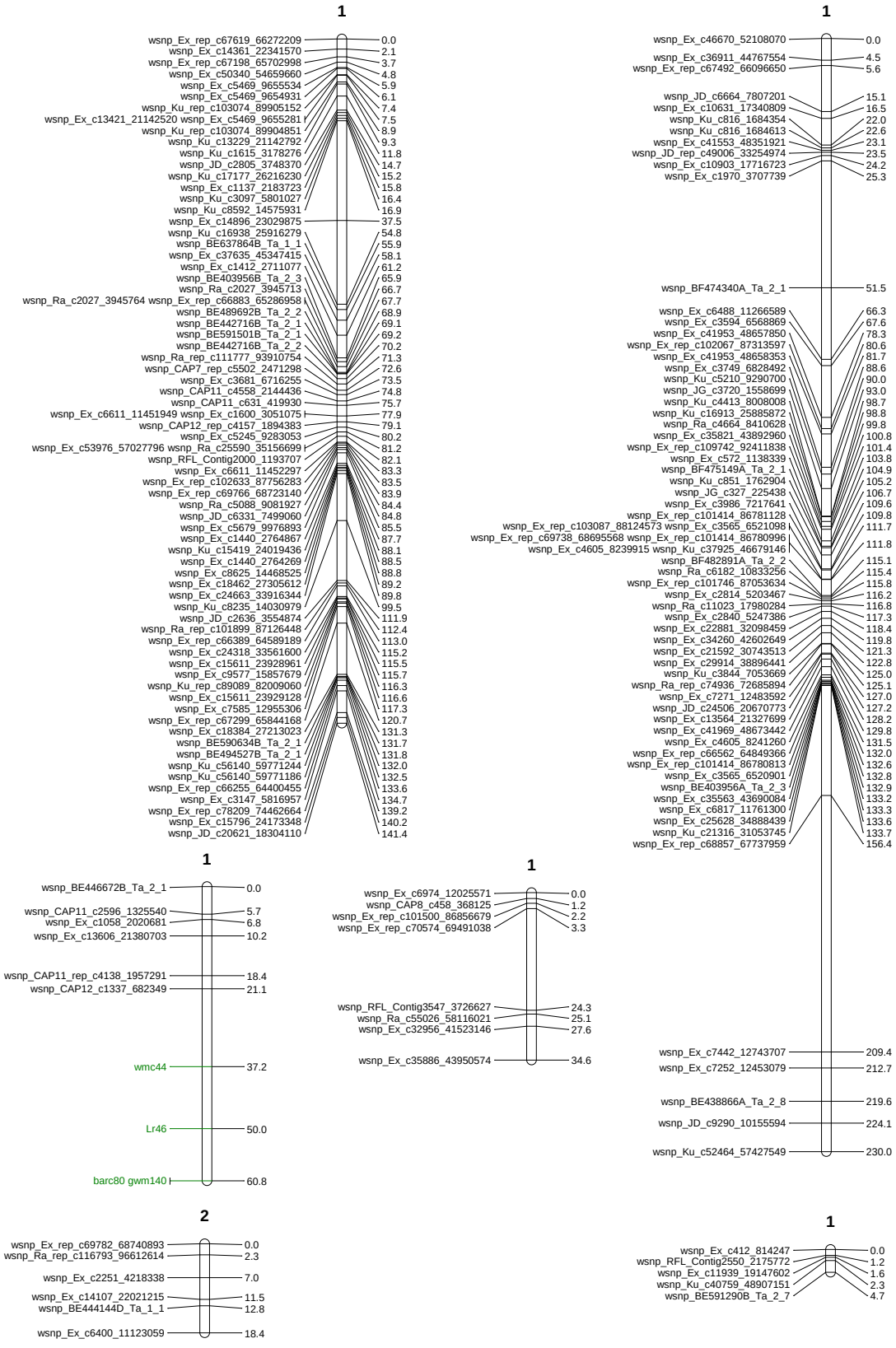
93E54*G46	14.885	3.865	15.000	5.557	20.333	7.984	70.000	6.893	65.000	-		-
93E54*G47	8.293	3.865	16.667	5.557	23.333	7.984	18.333	6.893	65.000		38.333	4.818
93E54*G48	3.641	3.865	5.333	5.557	3.323	9.249	40.000	6.893	45.000		20.000	4.818
93E54*G54	39.921	3.865	56.667	5.557	27.823	9.249	76.667	6.893	5.000		16.667	4.818
93E54*G58	11.555	3.865	8.333	5.557	15.333	7.984	43.333	6.893	45.000		26.667	4.818
93E54*G62	4.982	3.865	10.000	5.557	5.681	9.248	21.667	6.893	5.000		20.000	4.818
93E54*G63	2.337	3.865	10.000	5.557	5.333	7.984	15.000	6.893	0.000		15.000	4.818
93E54*G67	2.430	3.865	5.333	5.557	0.000	7.984	6.667	6.893	0.000		11.667	4.818
93E54*G7	8.235	3.865	1.000	5.557	16.667	7.984	76.667	6.893	65.000		28.333	4.818
93E54*G70	0.670	3.865	1.000	5.557	0.823	9.249	6.667	6.893	0.000		25.000	4.818
93E54*H10	26.708	3.865	28.333	5.557	31.667	7.984	76.667	6.893	5.000		18.333	4.818
93E54*H11	19.946	3.865	63.333	5.557	40.323	9.249	65.000	6.893	5.000		15.000	4.818
93E54*H23	40.013	3.865	70.000	5.557	42.681	9.248	80.000	6.893	75.000		70.000	4.818
93E54*H25	13.401	3.865	21.667	5.557	17.681	9.248	16.667	6.893	45.000		20.000	4.818
93E54*H26	30.048	3.865	36.667	5.557	37.823	9.249	53.333	6.893	65.000		46.667	4.818
93E54*H28	3.629	3.865	10.000	5.557	13.667	7.984	8.333	6.893	5.000		20.000	4.818
93E54*H5	5.326	3.865	6.667	5.557	18.333	7.984	26.667	6.893	65.000		20.000	4.818
93E54*H6	9.877	3.865	18.333	5.557	0.823	9.249	11.667	6.893	5.000		15.000	4.818

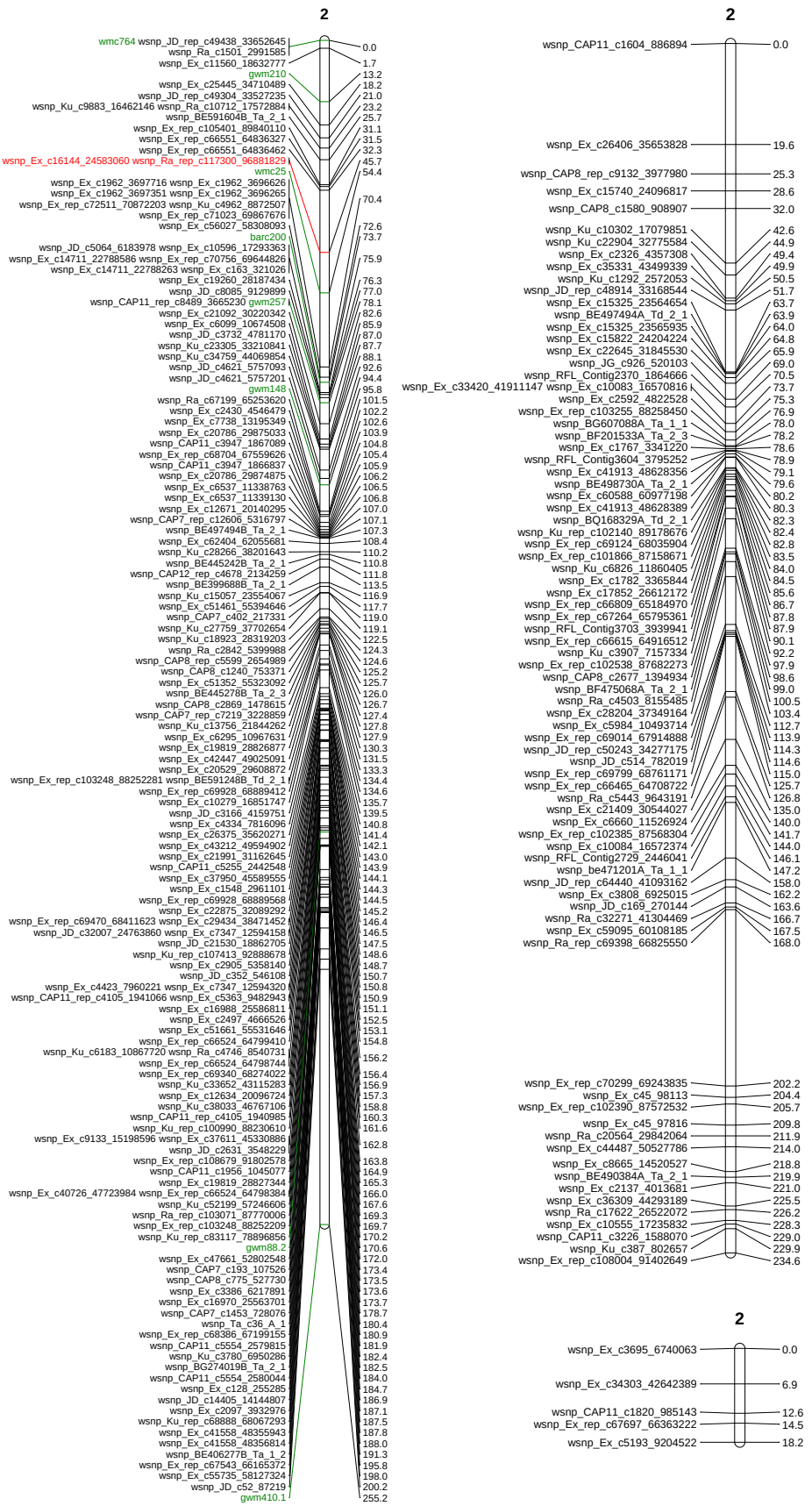
93E54*H8	23.447	3.865	23.333	5.557	21.667	7.984	61.667	6.893	75.000	65.000	4.818
93E54*H9	16.682	3.865	23.333	5.557	30.000	7.984	65.000	6.893	75.000	33.333	4.818
93E54*J10	3.549	3.865	6.667	5.557	11.667	7.984	11.667	6.893	0.000	15.000	4.818
93E54*J15	8.325	3.865	11.667	5.557	20.323	9.249	76.667	6.893	65.000	15.000	4.818
93E54*J20	6.714	3.865	10.000	5.557	31.008	12.282	58.333	6.893	5.000	16.667	4.818
93E54*J21	5.038	3.865	15.000	5.557	7.333	7.984	13.333	6.893	75.000	21.667	4.818
93E54*J3	14.905	3.865	23.333	5.557	23.333	7.984	51.667	6.893	5.000	25.000	4.818
93E54*J4	39.982	3.865	26.667	5.557	18.323	9.249	73.333	6.893	5.000	15.238	5.871
93E54*J8	0.713	3.865	5.333	5.557	1.000	7.984	8.333	6.893	0.000	11.667	4.818
93E54*J9	46.662	3.865	66.667	5.557	40.000	7.984	76.667	6.893	65.000	68.333	4.818
DH102	4.995	3.865	13.333	5.557	-1.819	9.248	7.000	6.893	0.000	15.000	4.818
DH102B	16.751	3.865	40.000	5.557	35.000	7.984	63.333	6.893	75.000	21.667	4.818
DH103	6.606	3.865	10.000	5.557	16.667	7.984	28.333	6.893	0.000	31.667	4.818
DH104	43.258	3.865	66.667	5.557	35.181	9.248	80.000	6.893	75.000	90.000	4.818
DH105B	6.642	3.865	13.333	5.557	0.681	9.248	71.667	6.893	0.000	73.333	4.818
DH109	0.534	3.865	3.667	5.557	1.000	7.984	3.667	6.893	15.000	30.000	4.818
DH122	43.375	3.865	76.667	5.557	46.667	7.984	80.000	6.893	85.000	83.333	4.818
DH124	8.240	3.865	8.333	5.557	22.681	9.248	55.000	6.893	15.000	28.333	4.818

DH135	23.477	3.865	21.667	5.557	50.000	7.984	73.333	6.893	75.000	38.333	4.818
DH136	1.049	3.865	3.667	5.557	-1.677	9.249	10.000	6.893	15.000	30.000	4.818
DH141	2.211	3.865	6.667	5.557	2.000	7.984	7.000	6.893	65.000	15.000	4.818
DH144	3.702	3.865	3.667	5.557	17.681	9.248	18.333	6.893	0.000	16.667	4.818
DH147	16.636	3.865	20.000	5.557	15.181	9.248	70.000	6.893	65.000	16.667	4.818
DH155	11.716	3.865	16.667	5.557	28.333	7.984	68.333	6.893	5.000	16.000	4.818
DH156A	19.943	3.865	23.333	5.557	32.681	9.248	70.000	6.893	45.000	28.333	4.818
DH159	5.372	3.865	13.667	5.557	13.333	7.984	30.000	6.893	45.000	15.000	4.818
DH90	5.538	3.865	8.333	5.557	22.681	9.248	20.000	6.893	0.000	16.667	4.818
DH91	3.287	3.865	8.333	5.557	18.667	7.984	46.667	6.893	0.000	26.667	4.818
DH95	21.698	3.865	56.667	5.557	21.667	7.984	76.667	6.893	45.000	80.000	4.818
LSD *P*<0.05	9.801		15.441		18.311		18.844		na	13.162	

[a]2011 Lethbridge stripe rust data was un-replicated therefore the reported values are the actual disease ratings for each ID in one replication. Note: "-" signifies missing data

Appendix 3. Genetic linkage map generated for the RL4452/AC Domain Population**.**

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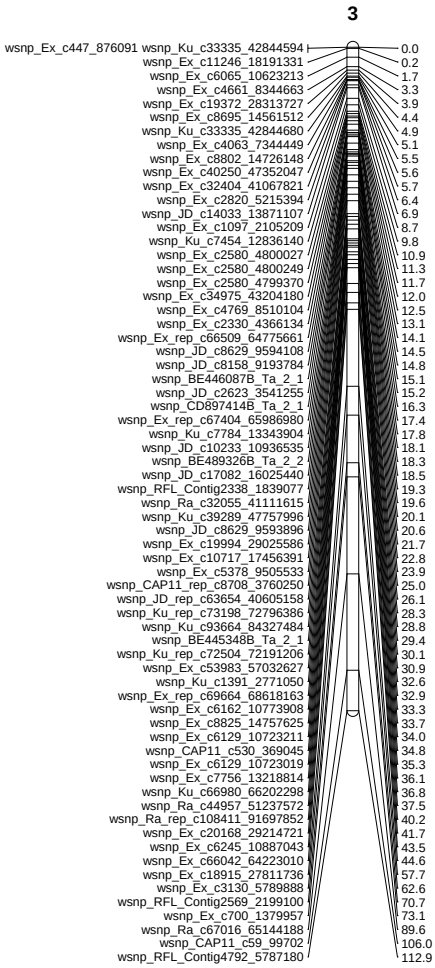
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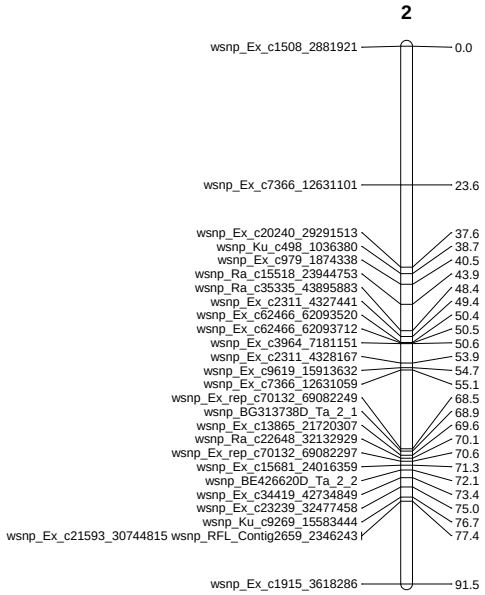
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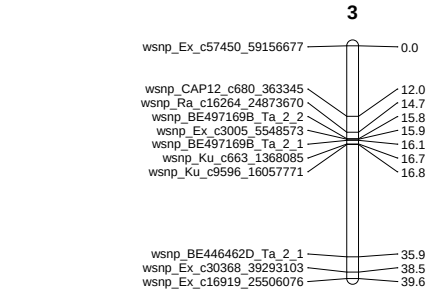
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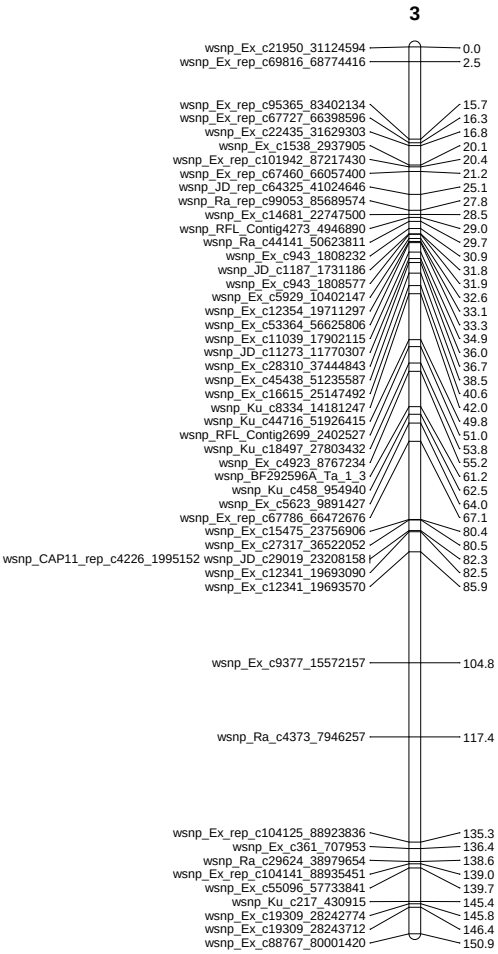
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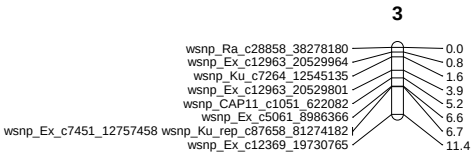
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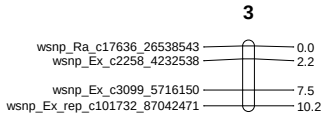
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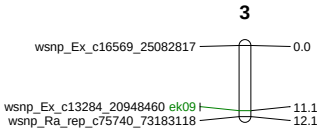
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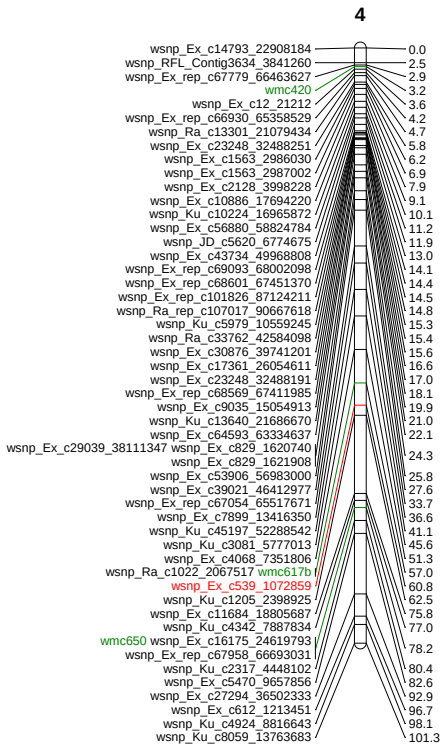
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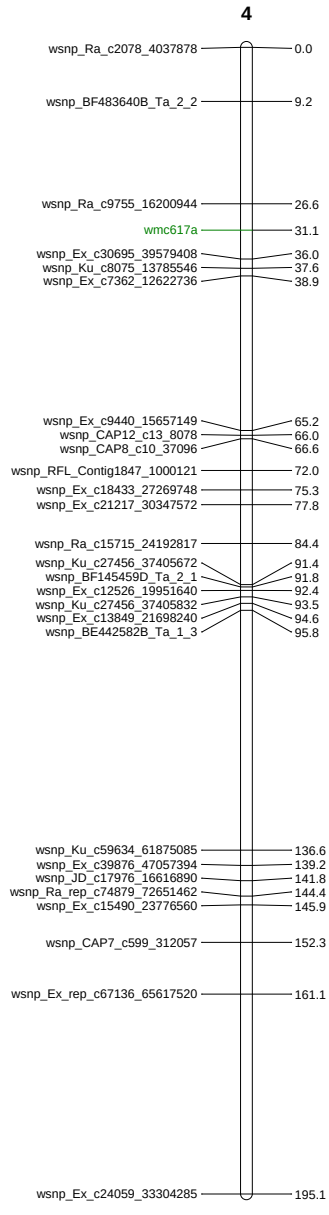
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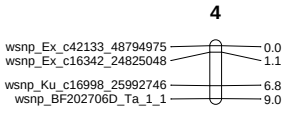
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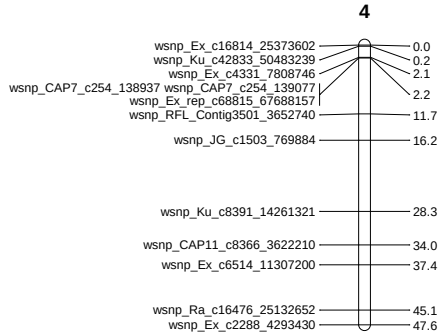
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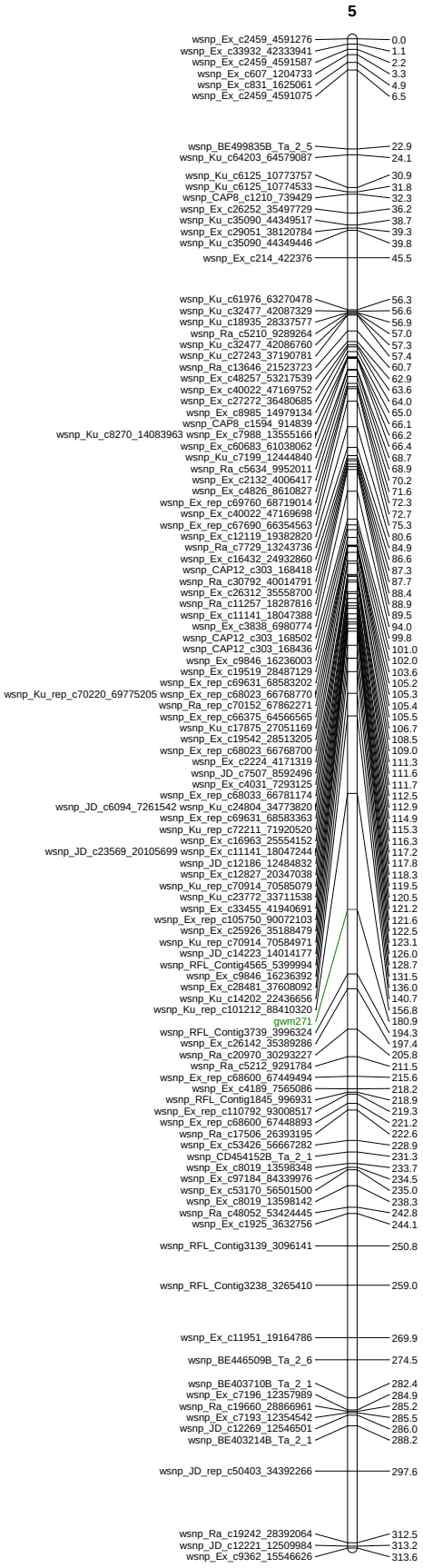
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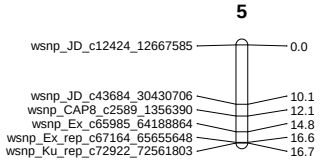
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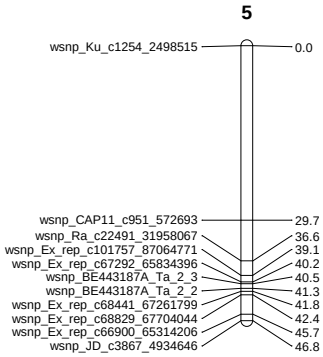
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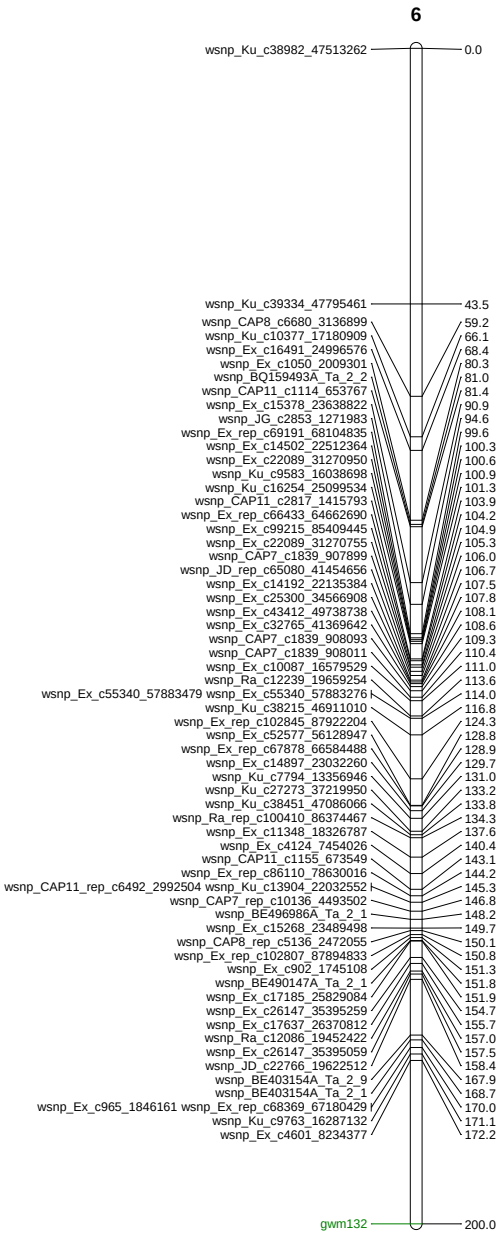
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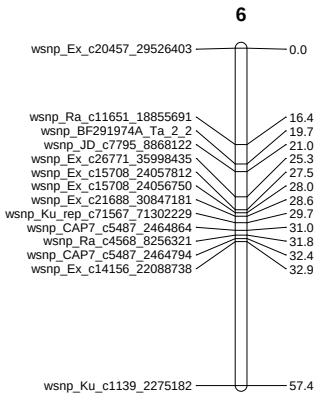
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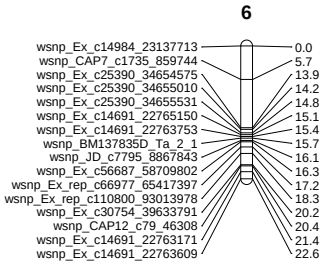
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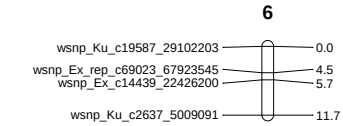
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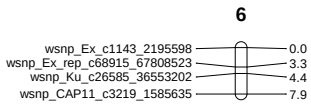
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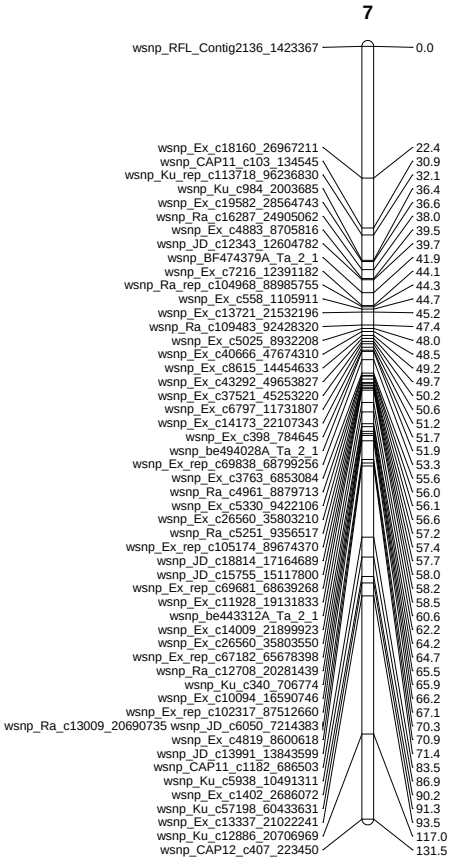
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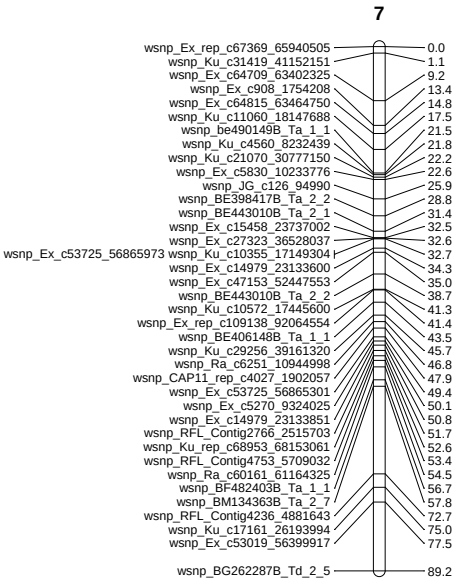
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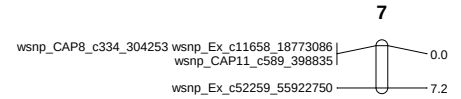
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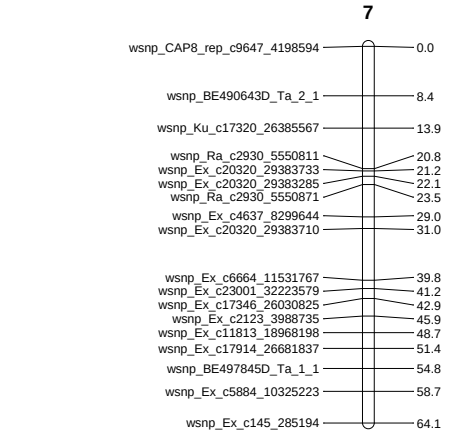
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wsnp_Ex_c2123_3988735 45.9
wsnp_Ex_c11813_18968198 48.7
wsnp_Ex_c17914_26681837 51.4
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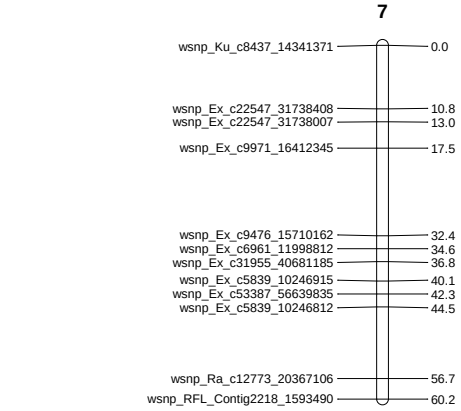
7
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wsnp_Ex_c6961_11998812 34.6
wsnp_Ex_c31955_40681185 36.8
wsnp_Ex_c5839_10246915 40.1
wsnp_Ex_c53387_56639835 42.3
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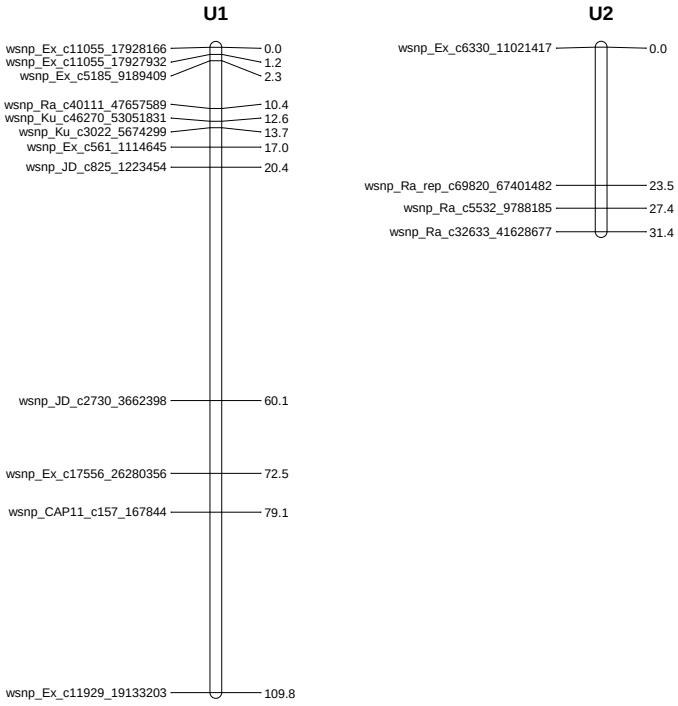
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wsnp_Ku_c3022_5674299
wsnp_Ex_c561_1114645
wsnp_JD_c825_1223454
wsnp_JD_c2730_3662398
wsnp_Ex_c17556_26280356
wsnp_CAP11_c157_167844
wsnp_Ex_c11929_19133203
0.0
1.2
2.3
10.4
12.6
13.7
17.0
20.4
60.1
72.5
79.1
109.8
U2
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wsnp_Ra_rep_c69820_67401482
wsnp_Ra_c5532_9788185
wsnp_Ra_c32633_41628677
0.0
23.5
27.4
31.4

Appendix 4. Primer Sequences designed from iSelect 9K probes associated with the peak of the major QTL: *Q.usw-2B1*

SNP- ***wsnp_Ex_c16144_24583060*****:**	**FF1-** GAAGGTGACCAAGTTCATGCTTTCCAGGCAATTATCT CATCTT **HFI-** GAAGGTCGGAGTCAACGGATTTTCCAGGCAATTATCT CATCTC **R-** GGTTGGATGGTGGTTTTCTG
SSCPSNP- ***wsnp_Ra_rep_c117300_96881829*****:**	**F-**TTCTTCTGAACCCGAAACAGAT **R-**TAGCAATGAGAATCTGAAGCCA

Appendix 5. Genotypic results for markers: SSCPSNP- *wsnp_Ra_rep_c117300_96881829, Lr34*, and *Lr46* run on the diversity panel.

Sample	***Q.usw-2B1***	***Lr34***	***Lr46***
AC Foremost	-	S	S
AC Vista	-	S	R
Burnside	-	R	R
BW878	-	S	S
BW880	-	S	S
BW883	-	R	S
Carberry	-	R	R
CDC Go	-	S	R
Choteau	-	S	R
Eurostar	-	R	R
Glencross VB	-	R	R
Outlook	-	R	R
Sadash	-	S	R
Unity GC	-	S	S
5602HR	R	R	S
5700PR	R	-	-

AC Abbey	R	-	-
AC Crystal	R	S	S
AC Eatonia	R	R	S
AC Infinity	R	S	S
AC Intrepid	R	S	R
AC Splendor	R	-	-
AC Taber	R	S	S
Alvena	R	S	R
CDC Kernen	R	R	R
CDC Alsask	R	R	S
CDC Osler	R	R	S
CDC Teal	R	R	S
CDC Zorba	R	S	R
CDC Zorba	R	-	-
Goodeve VB	R	S	R
GP003	R	S	R
NRG10	R	R	R
Helios	R	S	S
Conquer	R	R	R
Katepwa	R	-	-
Minnedosa	R	S	R
Muchmore	R	R	R
Prodigy	R	S	S
PT559	R	S	R
PT575	R	R	S
Red Fife	R	-	-
Roblin	R	-	-
Shaw VB	R	R	S
Snowstar	R	S	S

Waskada	R	S	S
03Spelt04	S	-	-
5600HR	S	-	-
5603HR	S	S	S
5701PR	S	-	-
5702PR	S	R	R
859CL	S	S	S
AC Avonlea	S	S	S
AC Barrie	S	-	-
AC Elsa	S	R	S
AC Morse	S	S	S
AC Navigator	S	S	R
AC Superb	S	S	S
Bhishaj	S	S	R
Brigade	S	S	S
CDC Abound	S	S	S
CDC Merlin	S	S	S
CDC Rama	S	R	R
CDC Verona	S	S	S
CDC Walrus	S	-	-
Cdn Bison	S	R	R
Commander	S	-	-
Enterprise	S	S	R
Fieldstar VB	S	S	S
Glenlea	S	R	R
Glenn	S	S	R
Harvest	S	-	-
Kane	S	-	-
Kyle	S	S	R

Lillian	S	R	S
McKenzie	S	S	S
Napoleon	S	S	S
Sandro	S	-	-
Snowbird	S	S	S
Somerset	S	S	S
Stettler	S	S	S
Strongfield	S	-	-
Marquis	U	-	-

U=Unique allele, "-" = missing data

Appendix 6: Variance estimates for random effects and F-values for fixed effects from analysis of variance (ANOVA) of percent disease severity (DS), area under the disease progress curve (AUDPC), coefficient of infection (CIS) and leaf tip necrosis (LTN) at the Saskatoon leaf rust nursery. Data was collected over 2011-2012 field seasons and was analyzed separately by year.

	DS		AUDPC		CIS		LTN	
	2011	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
Random Effect Variance Estimates								
Genotype(*Lr34*Lr46*Lr16*Q.usw-2B1*)	38.380***	91.786***	1488.71***	1304.99***	21.958***	94.502***	0.267***	0.155
Block(Rep)	0	0	28.836	22.782	0	0	0.073	0
Rep	6.854	0	188.20	5.133	3.157	0.165	0.075	0
Residual	35.971***	88.996***	112.88***	1180.03***	2.455***	91.739***	0.669***	1.807***
Fixed Effect F-Values								
Lr34	170.3***	76.37***	142.36***	90.58***	106.27***	64.94***	63.84***	24.15***
Lr46	3.95*	2.85	2.79	3.08	3.37	3.11	0.1	3.07
*Lr34*Lr46*	2.32	2.54	2.35	3.12	1.89	2.76	0.18	3.06
Lr16	15.59***	10.78*	16.86***	12.9**	13.22***	10.89*	0.46	5.98*
*Lr34*Lr16*	5.06*	0.72	5.27*	1.24	4.06*	0.66	0.36	0.8
*Lr46*Lr16*	1.56	1.21	2.43	1.69	1.49	1.93	1.94	0.07
*Lr34*Lr46*Lr16*	0.15	0.22	0.78	0.37	0.17	0.44	1.22	0.02
Q.usw-2B1	22.62***	42.36***	19.03***	35.94***	31.02***	43.31***	4.31*	15.31***
Lr34 Q.usw-2B1*	9.9***	10.77*	9.03**	6.44*	13.16***	13.32***	0.15	2.74
Lr46 Q.usw-2B1*	0.47	0.48	0.75	0.74	0.14	0.25	0.65	1.69
*Lr34*Lr46* Q.usw-2B1*	0.03	0.24	0.01	0.16	0	0.54	1.43	0.35
Lr16 Q.usw-2B1*	2.65	0.84	2.04	0.69	4.9*	0.33	5.29	0.06
*Lr34*Lr16* Q.usw-2B1*	0.21	0.18	0.31	0.17	0.9	0.49	0.17	0.02
*Lr46*Lr16* Q.usw-2B1*	0.17	0.55	0.03	0.55	0	0.26	0.5	0.37
*Lr34*Lr46*Lr16* Q.usw-2B1*	5.01*	3.4	3.23	2.77	3.18	3.43	4.42*	0.11

*P<0.05, **P<0.01, ***P<0.001

Appendix 7: Variance estimates for random effects and F-values for fixed effects from analysis of variance (ANOVA) of percent disease severity (DS) and coefficient of infection (CIP) at the Portage leaf rust nursery. Data was collected over 2011-2012 field seasons and was analyzed separately by year.

	DS		CIP	
	2011	**2012**	**2011**	**2012**
Random Effect Variance Estimates				
Genotype (*Lr34*Lr46*Lr16*Q.usw-2B1*)	33.109***	155.09***	13.630***	150.76***
Rep	65.623	4.948	15.957	8.554
Residual	125.12***	133.24***	52.997***	178.73***
Fixed Effect F-Values				
Lr34	118.92***	274.1***	37.09***	201.59***
Lr46	0.76	6.55*	0.31	6.05*
*Lr34*Lr46*	1.05	0.26	1.66	1.04
Lr16	0.04	0.02	0.09	1.23
*Lr34*Lr16*	0.68	3.78	0.16	4.14*
*Lr46*Lr16*	1.34	0.57	0.35	0.85
*Lr34*Lr46*Lr16*	0	2.33	0.92	2.53
Q.usw-2B1	17.64***	9.56**	6.86**	5.73*
*Lr34*Q.usw-2B1*	13.81**	0.31	6.33*	0.94
*Lr46*Q.usw-2B1*	0.05	0.15	0.66	0.51
*Lr34*Lr46*Q.usw-2B1*	0.01	4.96*	0.79	4.4*
*Lr16*Q.usw-2B1*	0.12	0.04	0.02	0.46
*Lr34*Lr16*Q.usw-2B1*	0.44	2.09	0.74	3.32
*Lr46*Lr16*Q.usw-2B1*	0.03	0.89	0.03	0.61
*Lr34*Lr46*Lr16*Q.usw-2B1*	0.03	0.67	0.07	0.15

*P<0.05, **P<0.01, ***P<0.001

Appendix 8: Variance estimates for random effects and F-values for fixed effects from analysis of variance (ANOVA) of percent disease severity (DS) for the Lethbridge stripe rust nursery. Data was collected over 2011-2012 field seasons and was analyzed separately by year.

	DS	
	2011	**2012**
Random Effect Variance Estimates		
Genotype(*Lr34*Lr46*Q.usw-2B1*)	681.02***	280.63***
Rep	-	3.036
Residual	0.999	70.739***
Fixed Effect F-Values		
Lr34	44.53***	43.48***
Lr46	2.47	1.36
*Lr34*Lr46*	0.82	0.26
Q.usw-2B1	1.67	1.78
*Lr34*Q.usw-2B1*	3.29	2.9
*Lr46*Q.usw-2B1*	1.35	2.6
*Lr34*Lr46*Q.usw-2B1*	0.07	1.54

*P<0.05, **P<0.01, ***P<0.001
Note: 2011 data was not replicated

Appendix 9: Variance estimates for random effects and F-values for fixed effects from analysis of variance (ANOVA) for LTN recorded at the Saskatoon testing environments. Data was collected over 2011-2012 field seasons and was analyzed separately by year.

	2011			2012		
	DS	AUDPC	CIS	DS	AUDPC	CIS
Random Effect Variance Estimates						
Rep (Block)	0	0	26.49	7.93	209.81*	6.65
Rep	15.95	8.46	425.94	0	0	0
Residual	154.82***	91.00***	5591.38***	223.86***	3220.79***	218.58***
Fixed Effect F-Values						
LTN	15.86***	19.07***	19.08***	38.75***	30.98***	40.62***

*P<0.05, **P<0.01, ***P<0.001